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Specification

Title of the invention

Thionucleoside Derivatives Having Potent Anticancer Activity and the Pharmaceutical Compositions Containing the Same

Detailed explanation of the invention

Objective of the invention

Technology of the invention and prior art

The objective of the present invention is to provide thionucleoside derivative compounds with useful novel chemical structures that have anti-cancer activities.

Adenosine is a compound that executes various physiological functions through specific receptors in cell membranes. Extracellular adenosine acts as a neurotransmitter in a variety of physiological systems. In general, adenosine counterbalances excessive activities of a given organ and thereby provides protection from the harmful effect of stress (Jacobson, K.A. et al.; J. Med. Chem., 35, pp. 407-422, 1992). This is a partially formed negative feedback loop in an attempt to decrease the cellular energy demand by adenosine formed via decomposition of endocellular and extracellular ATP (adenosine triphosphate) and to increase oxygen supply. Adenosine is important in maintaining the normality of essential organs such as the brain, heart and kidney. For example, it was proved that the injection of an adenosine agonist into the brain has a neuroprotective effect, and it is also known to be related to pain, intelligence, movement, and sleep.

Adenosine receptors have been classified as P1 and P2 receptors through pharmacological study and molecular cloning. Adenosine acts as a substrate for P1 receptors whereas ATP, ADP, UTP, and UDP act as substrates for P2 receptors to manifest physiological activity. Among these, four different subtypes of adenosine receptors were identified for P1 receptors, which were classified as A_1, A_2, or A_3 based on the ligand affinity, distribution in the system, and functional process. A_2 is again divided into A_{2a} and A_{2b}. These adenosine receptors is one class of G-protein-coupled receptors. The adenosine A_1, A_{2a} and A_{2b} receptors were pharmacologically identified using various selective ligands. However, the adenosine A_3 receptor was first identified in 1992 (Zhou, Q.Y. et al.; Proc. Natl. Acad. Sci. USA, 89, pp. 7432-7436, 1992). Numerous studies are being carried out to identify the particular physiological function of this receptor.

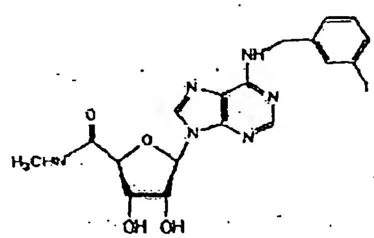
Adenosine A_1 and A_2 receptor agonists are usually antihypertensives, antipsychotic, anti-arrhythmia drugs, and fat metabolism inhibitors (diabetes treatment drugs), and neuroprotective drugs, which have been studied quite well. Antagonists are xanthine derivatives or compounds wherein various heterocycles are fused together, which have been developed for anti-asthmatics,

antidepressants, anti-arrhythmia drugs, renal protection drugs, anti-Parkinson's drugs, and nootropics. However, recently commercialized [sic; developed] drugs are adenosine itself for treatment of supraventricular tachycardia, and the adenosine transfer inhibiting drug, dipyridamole, which is being used as a supplemental drug for warfarin in preventing blood coagulation after heart surgery. Such development has been unsuccessful because adenosine receptors are present all over the system, and there are various concomitant pharmacological effects until the receptor is activated, and therefore no compound can activate only the adenosine receptor.

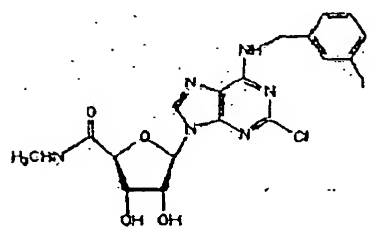
Among the adenosine receptors, the adenosine A_3 receptor has recently been identified as unlike the widely known adenosine receptors A_1 and A_2, and therefore its role has not yet been not elucidated. Various studies are in progress for development of selective ligands [for the A_3 receptor]. For pharmacological study on the adenosine A_3 receptor, three radiolabeled ligands such as [^{125}I]ABA (N^6-(4-amino-3-^{125}I[iodo]benzyl)adenosine), [^{125}I]APNEA ([^{125}I]N6-2-(4-aminophenyl)ethyl adenosine) or [^{125}I]AB-MECA ([^{125}I]4-aminobenzyl-5'-N-methylcarboxyamidoadenosine) are being employed. When A_3 receptor was expressed in Chinese hamster ovary (CHO) cells, it had an inhibiting effect on adenylyl cyclase, the enzyme that produces cAMP from ATP. When the A_3 receptor was activated by an agonist, it was proved that phosphatidyl inositol decomposed to activate GTP-dependent phospholipase C (guanosine triphosphate-dependent phospholipase), the enzyme that produces inositol phosphate and DAG (Ramkumar, V., et al.; J. Biol. Chem., 268, pp. 168871-168890, 1993: Abbracchio, M.P. et al.; Mol. Pharmacol., 48, pp. 1038-1045, 1995). This discovery explains the potential response pathway by A_3 activation for cerebral ischemia because this secondary transmitter system means the response pathway of nerve damage in brain ischemia. In addition, the adenosine A_3 agonist has a protective effect against brain diseases like epilepsy, and a protective effect for the heart. Activation of the adenosine A_3 receptor results in the emission of inflammation-inducing factor like histamine from mast cells, and contracts organs [sic]. High concentrations of agonist or antagonist may result in apoptosis in immune cells. An agonist of A_3 receptors inhibits the generation of tumor necrosis factor (TNF-α), which is the inflammation transmitter, and also inhibits the formation of MIP-1α, interleukin-12, and interferon-γ, which are inflammation mediators. Therefore, adenosine A_3 antagonists have the potential for development as anti-inflammatories and antiasthmatics. If a pharmocologically selective compound can be developed, development of new treatment drugs may be possible for various diseases like asthma, inflammation, cerebral ischemia, heart diseases, and cancer.

Among the compounds that have been developed and studied so far, N^6-(3-iodobenzyl)-5'-(N-methylcarbamoyl)adenosine* (IB-MECA) which is shown in the following Structure 1, and N^6-(3-iodobenzyl)-2-chloro-5'-(N-methylcarbamoyl)adenosine (CI-IB-MECA) shown in Structure 2 are representative selective adenosine A_3 ligands which showed high selectivity for A_3 receptors over adenosine A_1 and 2 receptors.

[Structure 1]



[Structure 2]



Analysis of various research results indicates that the N-methylcarbamoyl group must be present at the C5 position of the sugar component and, in the base, an arylamino group or alkylamino group must substitute on the C6 position of the purine to exhibit affinity toward A_3 receptors. No research has been reported on an affinity comparison study by introducing substituents other than the hydroxyl group at the C2 or C3 position of the sugar component. We have synthesized compounds with various substituents at the C2 or C3 position of the sugar component and have measured their affinity for adenosine A_3 receptors for [structure-activity] relationship study.

The ligands obtained from the structure-activity relationship study were chosen and their pharmacological study was carried out. The affinity for adenosine A_3 receptors and the selectivity for adenosine A_1 or A_2 receptors were measured, and a ligand that selectively interacts with the adenosine A3 receptor was developed. The present invention was completed by synthesizing

* [Editor's note: This compound name appears twice in the original document, once in Korean and once in English.]

thionucleoside derivatives that have no side effects and have increased pharmacological effect, and that are effective as anti-inflammatory and anticancer drugs.

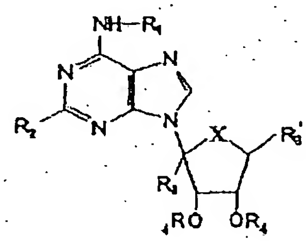
Technical task of the invention

The present invention offers thionucleoside derivative compounds with novel chemical structures that are useful for the treatment of cancer and inflammatory diseases.

Structure of the invention

To achieve the aforementioned objective, the present invention offers thionucleoside derivative compounds shown by the following general formula (1), their pharmacologically acceptable salts, and their isomers.

[Structure 3]



(I)

In the above formula,

X is sulfur or oxygen;

R_1 is hydrogen, alkyl group with 1-5 carbons, benzyl group, halobenzyl, phenylalkyl group;

R_2 is hydrogen, halogen group, alkoxy group, alkenyl group, alkynyl group, alkylthio group, or thio group;

R_3 and R_3' is hydroxyalkyl group with 1-5 carbons, alkoxycarbonyl group, or alkylaminocarbonyl group with 1-5 carbons, whereas R_3 and R_3' do not have identical substituents simultaneously;

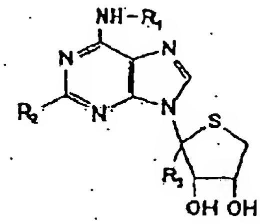
R_4 is hydrogen or alkyl group with 1-5 carbons.

The present invention also includes a pharmaceutical composition containing the compound of the aforementioned general formula (I) or its pharmacologically acceptable salt as an active ingredient.

The compound of the aforementioned general formula (I) wherein R_1 is 3-iodobenzyl group, R_2 is chloride, R_3 is methylaminocarbonyl group, R_3' and R_4 are hydrogen, and X is sulfur, includes the compounds of the following general formula (II) and its isomers, preferably

(2R, 3S,4R)-2-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-3,4-dihydroxytetrahydrothiophene-2-carboxylic acid methylamide.

[Structure 4]



(II)

The compound of the aforementioned general formula (I) in whicih R_1 is hydrogen, methyl group, or 3-iodobenzyl group, R_2 is chloride, R_3 and R_4 are hydrogen, R_3' is methylaminocarbonyl group or hydroxymethyl group, and X is sulfur, includes the compound of the following general formula (III) and its isomers, preferably:

(2R,3R,4S,5R)-2-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-3,4-dihydroxymethyltetrahydrothiopene-3,4-diol,

(2R,3R,4S,5R)-2-(2-chloro-6-methylaminopurin-9-yl)-5-hydroxymethyltetrahydrothiopene-3,4-diol,

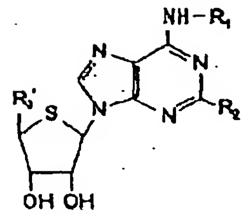
(2R,3R,4S,5R)-2-(2-chloro-6-methylaminopurin-9-yl)-5-hydroxymethyltetrahydrothiopene-3,4-diol,

(2S,3S,4R,5R)-5-(6-amino-2-chloro-purin-9-yl)-3,4-dihydroxytetrahydrothiopene-2-carboxylic acid methylamide,

(2S,3S, 4R,5R)-5-(2-chloro-6-methylaminopurin-9-yl)-3,4-dihydroxytetrahydrothiopene-2-carboxylic acid methylamide,

and (2S,3S, 4R,5R)-5-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-3,4-dihydroxytetrahydrothiopene-2-carboxylic acid methylamide.

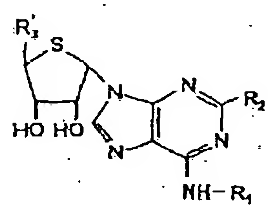
[Structure 5]



(III)

The compound of the aforementioned general formula (I) wherein R_1 is 3-iodobenzyl group, R_2 is chloride, R_3' is hydroxymethyl group, R_3 and R_4 are hydrogen, and X is sulfur, includes the compounds of the following general formula (IV) and its isomers, preferably (2S,3R,4S,5R)-2-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-5-hydroxymethyltetrahydrothiopene-3,4-diol.

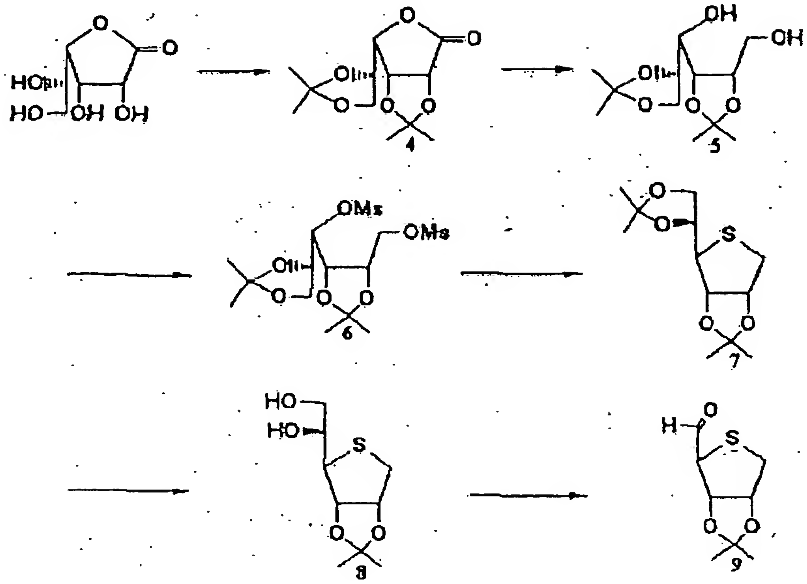
[Structure 6]



(IV)

Another objective of the present invention is to provide a manufacturing method for compounds with the aforementioned general formula (I), which can be chemically synthesized by the method as illustrated in the following reaction equations 1-7. However, the synthetic method is not limited to these examples. The following reaction equations list each step of the manufacturing method of the representative compounds of the present invention.

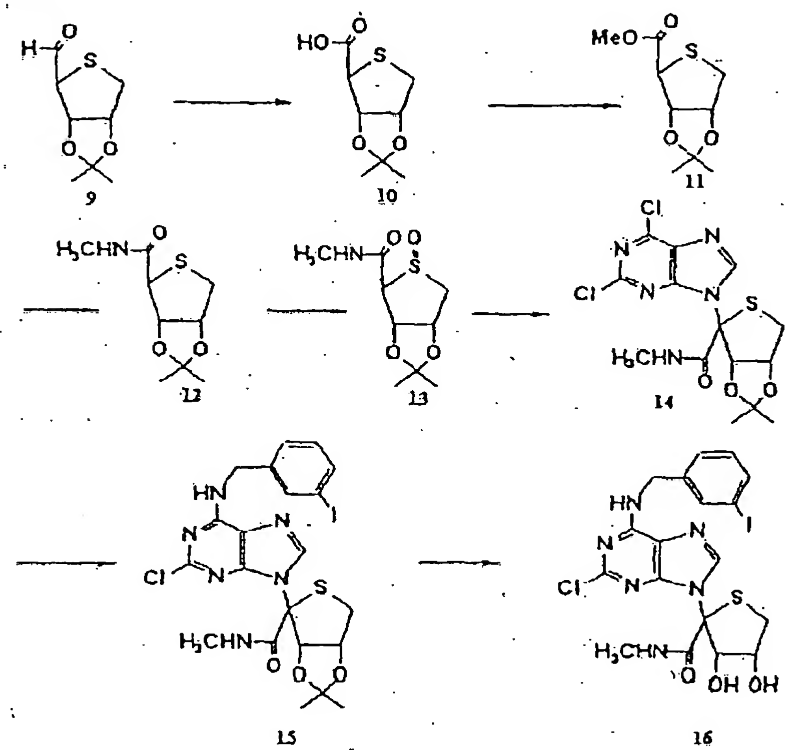
[Reaction equation 1]



As shown in the aforementioned reaction equation 1, the compound with chemical formula 4 can be synthesized from D-gulonic γ-lactone by reacting with dry acetone as a reagent and solvent at the same time using concentrated sulfuric acid as an acid catalysis in the presence of anhydrous copper(II) sulfate. This reaction can employ an inorganic acid such as hydrochloric acid or an organic acid such as p-toluenesulfonic acid as an acid catalyst instead of the concentrated sulfuric acid. As a dehydrating agent, it is possible to use a molecular sieve or anhydrous magnesium sulfate in addition to anhydrous copper(II) sulfate. The synthesized compound 4 was reacted with lithiumaluminum hydride to obtain compound 5. It is preferable to use an inert solvent such as ethyl ether, petroleum ether, dichloromethane, or tetrahydrofuran. It is also possible to use a metal hydride such as sodium borohydride instead of lithiumaluminum hydride. Compound 5 synthesized herein was reacted with methanesulfonyl chloride to obtain compound 6. It is preferable to use an amine reagent such as pyridine or triethylamine as a solvent and reagent at the same time, or in a mixture with an inert solvent such as ethyl ether, petroleum ether, dichloromethane, tetrahydrofuran, or N,N-dimethylformamide. As a reaction catalyst, N,N-dimethylaminopyridine or 2,6-lutidine may be used. A compound with the aforementioned chemical formula 7 can be obtained by reacting compound 6 with sodium sulfide, and it is also possible to use a sodium alkoxide after substitution reaction with a thioester like metal thioacetate, instead of sodium sulfide. It is preferred to use N,N-dimethylformamide and dimethyl sulfoxide as a solvent. It is also possible to react in a low-molecular-weight alcohol such as methanol or ethanol, which may be mixed with water or an inert organic solvent.

Compound 7 synthesized herein was reacted with an aqueous acetic acid solution to obtain compound 8. Instead of acetic acid, an inorganic acid such as sulfuric acid or hydrochloric acid or an organic acid such as p-toluenesulfonic acid can be used with water or a low-molecular-weigh alcohol like methanol alone as a solvent or as a mixture with an organic solvent. Compound 9 with aforementioned chemical formula 9 can be obtained by reacting compound 8 with lead tetraacetate, wherein it is possible to use sodium metaperiodate at a low temperature instead of lead tetraacetate. It is preferred to use an inert solvent such as ethyl acetate, ethyl ether, petroleum ether, dichloromethane, tetrahydrofuran, or N,N-dimethylformamide.

[Reaction equation 2]



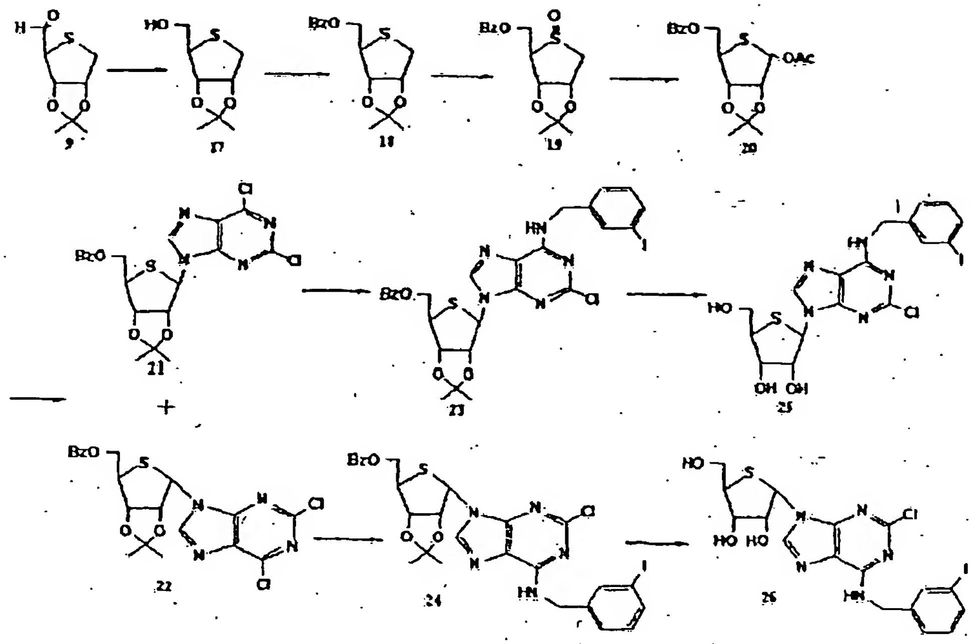
As shown in the above reaction equation 2, the compound with the aforementioned chemical formula 10 can be obtained by reacting compound 9 from reaction equation 1 with pyridinium dichloromate in N,N-dimethylformamide solvent. The compound of chemical formula 11 can be obtained by reacting the compound of chemical formula 10 with dimethyl sulfate in the presence of potassium carbonate, wherein it is possible that dimethyl sulfate can be replaced with a methyl halide such as methyl iodide or diazomethane, and potassium carbonate can be replaced with an inorganic base such as sodium carbonate or an organic base such as DBU

or n-butyllithium. It is also possible to use acetone as a solvent, or an organic solvent such as tetrahydrofuran or dioxane.

The compound with chemical formula 12 can be obtained by the reaction of the compound with chemical formula 11 with methylaminetetrahydrofuran solution or aqueous solution. As a solvent, tetrahydrofuran alone can be used or water can be mixed with an inert solvent such as ethyl ether, petroleum ether, dichloromethane, tetrahydrofuran, or N,N-dimethylformamide. The compound of chemical formula 13 can be obtained by the reaction of the compound of chemical formula 12 with m-chloroperbenzoic acid, wherein it is possible to use sodium metaperiodate, t-butylperoxide, peracetic acid, or hydrogen peroxide instead of m-chloroperbenzoic acid. As a solvent, it is preferred that an inert solvent such as ethyl ether, petroleum ether, dichloromethane, tetrahydrofuran, N,N-dimethylformamide be used alone, or a mixed solvent of these inert solvents and water may be used.

The compound with chemical formula 14 can be obtained by reacting a compound having chemical formula 13 with silylated 2,6-dichloropurine in the presence of trimethylsilyl trifluoromethanesulfonate, wherein an inert solvent such as dichloroethane, chloroform, acetonitrile, or dichloromethane is preferred as a solvent. Silylated 2,6-dichloropurine can be prepared by the reaction of hexamethyldisilazane with 2,6-dichloropurine as a reagent and solvent in the presence of ammonium sulfate catalyst, or by the direct reaction of 2,6-dichloropurine with N,O-bis(trimethylsilyl)acetamide. The compound having chemical formula 15 was obtained by reacting the compound having chemical formula 14 with 3-iodobenzylamine hydrochloride in the presence of triethylamine base. Instead of triethylamine, an organic base such as pyridine, N,N-dimethylaminopyridine or 2,6-lutidine can be used. It is preferred to use a low-molecular-weigh alcohol like methanol or ethanol, 1,4-dioxane, tetrahydrofuran, or chloroform as a solvent. The compound having chemical formula 16 was prepared by reacting the compound having chemical formula 15 with an aqueous solution of acetic acid, wherein an inorganic acid such as sulfuric acid or hydrochloric acid or an organic acid such as p-toluenesulfonic acid may be substituted for the acetic acid, which can be mixed with water alone or a low-molecular-weight alcohol alone such as methanol as a solvent, or with a mixed solvent of water or low-molecular-weight alcohol and organic solvent.

[Reaction equation 3]



As shown in the above reaction equation 3, the compound having chemical formula 17 can be obtained by reacting the compound having chemical formula 9 with sodium borohydride, wherein it is preferred to use a low-molecular-weight alcohol such as methanol or ethanol, and it is also possible to carry out the reaction in a mixed solvent of these low-molecular-weight alcohols and dichloromethane, tetrahydrofuran, or chloroform. The compound having chemical formula 18 can be obtained by reacting the compound having chemical formula 17 with benzoyl chloride, wherein it is preferred to use an amine reagent like pyridine or triethylamine as both a solvent and reagent or by mixing with an inert solvent such as ethyl ether, petroleum ether, dichloromethane, tetrahydrofuran, N,N-dimethylformamide, or chloroform. As a reaction catalyst, N,N-dimethylaminopyridine or 2,6-lutidine may be used.

The compound having chemical formula 19 can be obtained by reacting the compound having chemical formula 18 and m-chlorobenzoic acid. Instead of m-chlorobenzoic acid, sodium metaperiodate, t-butylperoxide, peracetic acid, or hydrogen peroxide may be used. It is preferred to use an inert solvent alone such as ethyl ether, petroleum ether, dichloromethane, tetrahydrofuran, N,N-dimethylformamide, or to use a mixed solvent of an inert solvent and water. The compound having chemical formula 20 was obtained by reacting the compound having chemical formula 19 with acetic anhydride, wherein the acetic anhydride is preferred to be used as both a solvent and a reagent. In addition, it is desirable to increase the reactivity by adding an acetate ion such as sodium acetate or tetrabutylammonium acetate.

Each of the compounds having chemical formulas 21 and 22 can be obtained by reacting the compound having chemical formula 20 with silylated 2,6-dichloropurine in the presence of trimethylsilyl trifluoromethanesulfonate. As a solvent, it is preferred to use an inert solvent such as dichloroethane, chloroform, acetonitrile, or dichloromethane. The silylated 2,6-dichloropurine can be obtained by reacting 2,6-dichloropurine with hexamethyldisilazane used as both a solvent and a reagent in the presence of ammonium sulfate catalyst, or by direct reaction of 2,6-dichloropurine with N,O-bis(trimethylsilyl)acetamide. Instead of trimethylsilyl trifluoromethanesulfonate, an inorganic catalyst such as tin(IV) chloride may be used.

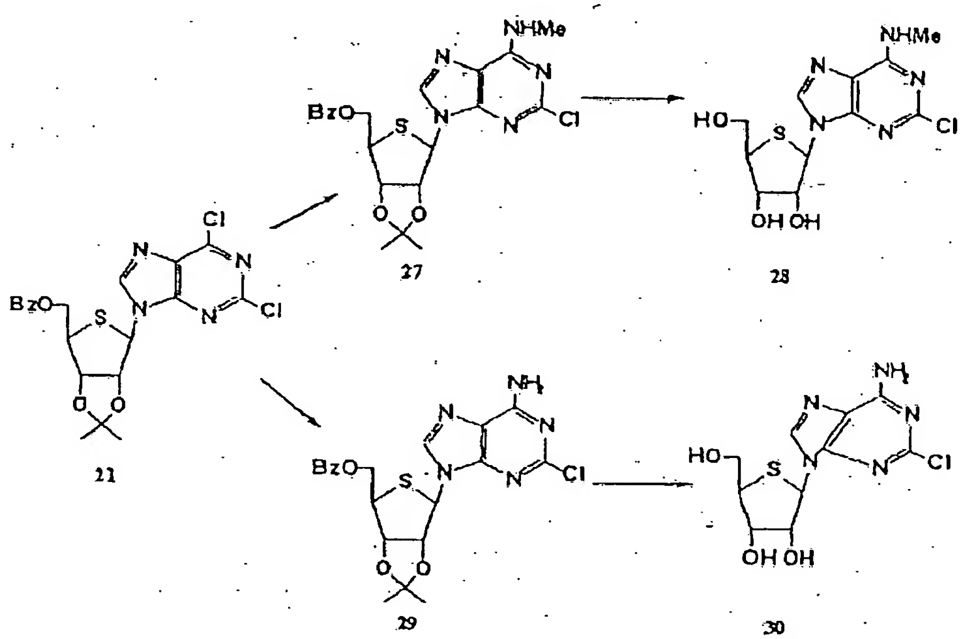
The compound having chemical formula 23 can be obtained by reacting the compound having chemical formula 21 with 3-iodobenzylamine hydrochloride in the presence of triethylamine base, wherein triethylamine can be replaced with an organic base such as pyridine, N,N-dimethylaminopyridine or 2,6-lutidine. As a solvent, it is preferred to use 1,4-dioxane, tetrahydrofuran, chloroform, or a low-molecular-weight alcohol such as methanol or ethanol. The compound having chemical formula 24 can be prepared by reacting the compound having chemical formula 22 with 3-iodobenzylamine hydrochloride in the presence of triethylamine base. Instead of triethylamine, an organic base such as pyridine, N,N-dimethylaminopyridine, or 2,6-lutidine may be used. As a solvent, 1,4-dioxane, tetrahydrofuran, chloroform, or a low-molecular-weight alcohol such as methanol or ethanol is preferred.

The compound having chemical formula 25 can be obtained by treating the resulting compound prepared in the following method with an aqueous acetic acid solution: by reacting the compound having chemical formula 23 with a metal alkoxide such as sodium methoxide or sodium ethoxide in a low-molecular-weight alcohol such as methanol or ethanol; by reacting the compound having chemical formula 23 with ammonia in an alcohol solvent such as methanol or ethanol; or by reacting the compound having chemical formula 23 with an inorganic base such as sodium carbonate or sodium hydrogen carbonate in a mixed solvent of water and a low-molecular-weight alcohol. The acetic acid may be substituted with an inorganic acid such as sulfuric acid or hydrochloric acid or an organic acid such as p-toluenesulfonic acid. As a solvent, it is also possible to use water alone or a low-molecular-weight alcohol like methanol alone or a mixed solvent with an organic solvent.

The compound having chemical formula 26 can be obtained by reacting an aqueous solution of acetic acid with the compounds resulting from the following methods: by reacting the compound having chemical formula 24 with a metal alkoxide such as sodium methoxide or sodium ethoxide in a low-molecular-weight alcohol solvent such as methanol or ethanol or in a mixed solvent with an inert solvent like dichloromethane or chloroform; by reacting the compound having chemical formula 24 with ammonia in an alcohol solvent such as methanol or ethanol; or by reacting the compound having chemical formula 24 with an inorganic base such as

sodium carbonate or sodium hydrogen carbonate in a mixed solvent of water and a low-molecular-weight alcohol. Instead of the acetic acid, an inorganic acid like sulfuric acid or hydrochloric acid or an organic acid such as p-toluenesulfonic acid may be used in water alone or a low-molecular-weight alcohol alone such as methanol, or in a mixed solvent with an organic solvent.

[Reaction equation 4]



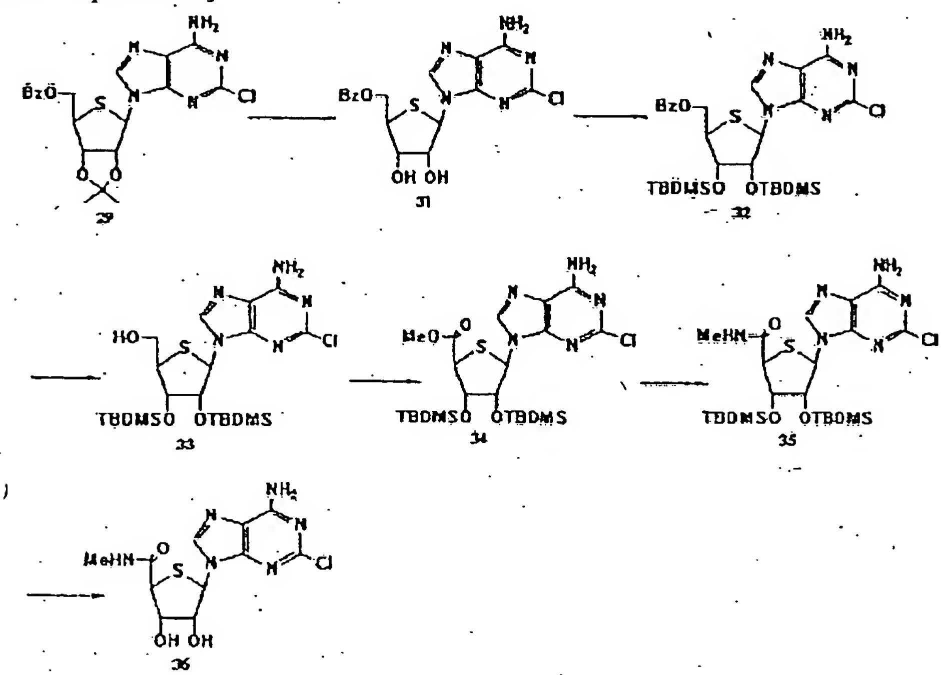
As shown in the above reaction equation 4, the manufacturing method of the compound having general formula (III) and the thionucleoside compound from the compound having chemical formula 21 as a starting material is described in reaction equation 4. R_1 is a hydroxymethyl group, R_2 is a methylamino group, and R_3 is a chloro group for the compound having the general formula (III), whereas R_1 is a hydroxymethyl group, R_2 is an amine group, and R_3 is a chloro group for the thionucleoside compound.

The compound having chemical formula 27 can be obtained by reacting the compound having chemical formula 21 with methylamine-tetrahydrofuran solution or an aqueous solution of methylamine. It is preferred to carry out the reaction in a closed container of metal or a special material. The compound having chemical formula 28 may be obtained from the compound resulting from the reaction of the compound having chemical formula 27 with an aqueous solution of acetic acid using the following methods, wherein an inorganic acid such as sulfuric acid or hydrochloric acid or an organic acid like p-toluenesulfonic acid may be used instead of

acetic acid, in water or a low-molecular-weight alcohol alone as a solvent or in a mixed solvent with an organic solvent; by reacting with a metal alkoxide such as sodium methoxide or sodium ethoxide in a low molecular weight solvent like methanol or ethanol, or in a mixed solvent with an inert solvent such as dichloromethane or chloroform; by reacting with ammonia in an alcohol solvent like methanol or ethanol; or by reacting with an inorganic base like sodium carbonate or sodium hydrogen carbonate in a mixed solvent of water and a low-molecular-weight alcohol.

The compound having chemical formula 29 can be obtained by reacting the compound having chemical formula 21 with a saturated ammonia solution in a low-molecular-weight alcohol such as ethanol or methanol. It is also preferred to react with a saturated ammonia solution in 1,4-dioxane. The compound having chemical formula 30 can be obtained by the following methods from the compound resulting from the reaction of the compound having chemical formula 29 with an aqueous solution of acetic acid, or an inorganic acid such as sulfuric acid or hydrochloric acid or an organic acid like p-toluenesulfonic acid can be used instead of acetic acid, in water or a low-molecular-weight alcohol such as methanol alone, or in a mixed solvent with an organic solvent; by reacting with a metal alkoxide such as sodium methoxide or sodium ethoxide in a low-molecular-weight solvent or in a mixed solvent with an inert solvent such as dichloromethane or chloroform, by reacting with ammonia in an alcohol solvent such as methanol or ethanol, or by reacting with an inorganic base such as sodium carbonate or sodium hydrogen carbonate in a mixed solvent of water and a low-molecular-weight alcohol.

[Reaction equation 5]



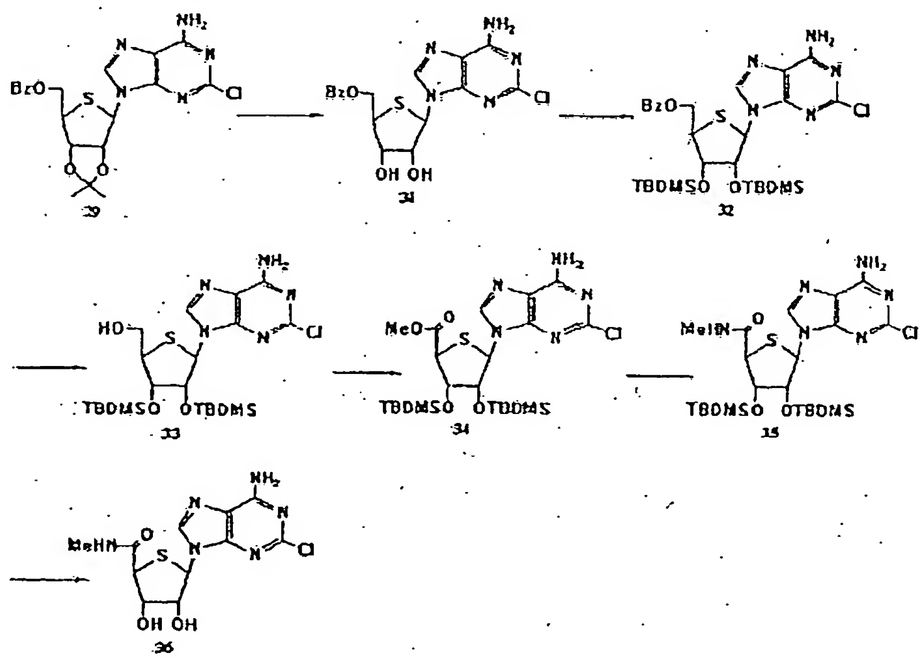
As shown in the reaction equation 5 above, the compound having chemical formula 31 can be obtained from the reaction of the compound having chemical formula 29 with acetic acid, wherein an inorganic acid like sulfuric acid or hydrochloric acid or an organic acid such as p-toluenesulfonic acid may be used instead of acetic acid, in water or a low-molecular-weight alcohol such as methanol alone, or in a mixed solvent with an organic solvent. The compound having chemical formula 32 can be prepared by reacting the compound having chemical formula 31 with tert-butylchlorodimethylsilane in the presence of imidazole base, wherein an organic base such as triethylamine, pyridine, N,N-dimethylaminopyridine or 2,6-lutidine may be used instead of imidazole, and it is preferred to use an inert solvent such as N,N-dimethylaminoformamide, dichloromethane, tetrahydrofuran, or chloroform as a solvent.

The compound having chemical formula 33 can be obtained by reacting the compound having chemical formula 32 with a metal alkoxide such as sodium methoxide or sodium ethoxide in a low-molecular-weight alcohol such as methanol or ethanol or in a mixed solvent with an inert solvent like dichloromethane or chloroform; by reacting with ammonia in an alcohol solvent such as methanol or ethanol; or by reacting with an inorganic base such as sodium carbonate or sodium hydrogen carbonate in a mixed solvent of water and a low-molecular-weight alcohol. The compound having chemical formula 34 can be obtained by reacting dimethyl sulfate and potassium carbonate with the compound resulting from the reaction of the compound having

chemical formula 33 with pyridinium dichromate in N,N-dimethylformamide solvent. Diazomethane or a methyl halide like methyl iodide may be used instead of dimethyl sulfate, whereas an inorganic base such as sodium carbonate or an organic base like DBU or n-butyllithium may be substituted for potassium carbonate. It is also possible to use acetone as a solvent or to use an organic solvent like tetrahydrofuran or dioxane.

The compound having chemical formula 35 can be obtained by reacting the compound having chemical formula 34 with methylamine solution in tetrahydrofuran or in 'water, wherein tetrahydrofuran alone may be used as a solvent or an inert solvent, like ethyl ether, petroleum ether, dichloromethane, tetrahydrofuran, or N,N-dimethylformamide may be mixed with water as a solvent. The compound having chemical formula 36 can be prepared by reacting the compound having chemical formula 35 with tetrabutylammonium fluoride, wherein a reagent that can generate a fluoride, such as triethylamine trihydrofluoride or hydrogen fluoride-pyridine, may be used instead of tetrabutyl ammonium fluoride. It is also desirable to use an organic acid like p-toluenesulfonic acid or an inorganic acid such as hydrochloric acid.

[Reaction equation 6]



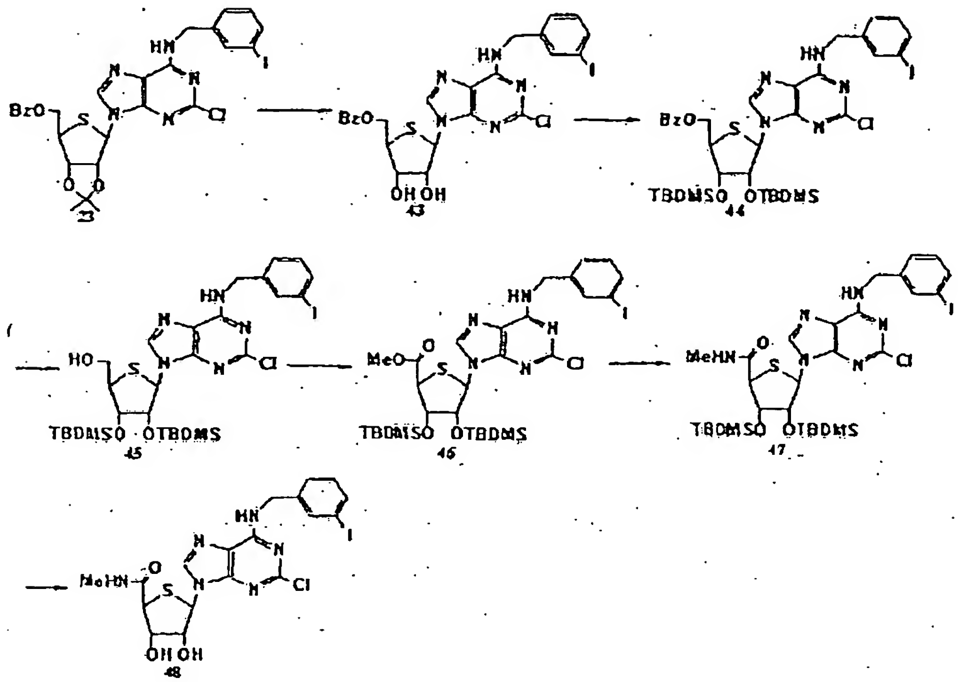
As shown in reaction equation 6, the compound having chemical formula 37 can be obtained by reacting the compound having chemical formula 27 with an aqueous solution of acetic acid, or with an inorganic acid such as sulfuric acid or hydrochloric acid or an organic acid

like p-toluenesulfonic acid instead of acetic acid, in water or a low-molecular-weight alcohol like methanol alone, or in a mixed solvent with an organic solvent. The compound having chemical formula 38 can be obtained by reacting the compound having chemical formula 37 with tert-butylchlorodimethylsilane in the presence of imidazole base, wherein an organic base such as triethylamine, pyridine, N,N-dimethylaminopyridine, or 2,6-lutidine may be used instead of imidazole. It is preferred to use an inert solvent like N,N-dimethylaminoformamide, dichloromethane, tetrahydrofuran, or chloroform as a solvent.

The compound having chemical formula 39 can be prepared by reacting the compound having chemical formula 38 with a metal alkoxide such as sodium methoxide or sodium ethoxide in a low-molecular-weight alcohol such as methanol or ethanol, or in a mixed solvent with an inert solvent such as dichloromethane or chloroform; with ammonia in an alcohol solvent such as methanol or ethanol; or with an inorganic base such as sodium carbonate or sodium hydrogen carbonate in a mixed solvent of water and a low-molecular-weight alcohol. The compound having chemical formula 40 can be obtained by reacting dimethyl sulfate and potassium carbonate with the resulting compound from the reaction of the compound having chemical formula 39 with pyridinium dichromate in N,N-dimethylformamide. It is possible to use a methyl halide like methyl iodide or diazaomethane instead of dimethyl sulfate, whereas potassium carbonate may be replaced with an inorganic base such as sodium carbonate or an organic base such as DBU or n-butyllithium. Acetone may be used as a solvent or it is also possible to use an organic solvent like tetrahydrofuran or dioxane.

The compound having chemical formula 41 may be obtained from the reaction of the compound having chemical formula 40 with methylamine in tetrahydrofuran solution or in aqueous solution, wherein tetrahydrofuran alone may be used as a solvent, or an inert solvent such as ethyl ether, petroleum ether, dichloromethane, tetrahydrofuran, or N,N-dimethylformamide may be mixed with water as the solvent. The compound having chemical formula 42 can be obtained from the reaction of the compound having chemical formula 41 with tetrahydrobutylammonium fluoride. A reagent that can generate a fluoride such as triethylamine trihydrofluoride or hydrogen fluoride-pyridine may be used instead of tetrahydrobutylammonium fluoride, or it is also desirable to use an organic acid such as p-toluenesulfonic acid or an inorganic acid like hydrochloric acid.

[Reaction equation 7]



As shown in the reaction equation 7 above, the compound having chemical formula 43 can be obtained by reacting the compound having chemical formula 23 with acetic acid or with an inorganic acid such as sulfuric acid or hydrochloric acid, or an organic acid like p-toluenesulfonic acid instead of acetic acid, in water or a low-molecular-weight alcohol such as methanol alone or in a mixed solvent with an organic solvent. The compound having chemical formula 44 may be obtained by reacting the compound having chemical formula 43 with tert-butylchlorodimethylsilane in the presence of imidazole base, wherein an organic base such as triethylamine, pyridine, N,N-dimethylaminopyridine or 2,6-lutidine may also be used instead of imidazole. It is desirable to use an inert solvent like N,N-dimethylaminoformamide, dichloromethane, tetrahydrofuran, or chloroform as a solvent. The compound having chemical formula 45 can be obtained by reacting the compound having chemical formula 44 with a metal alkoxide such as sodium methoxide or sodium ethoxide in a low-molecular-weight alcohol

solvent such as methanol or ethanol or in a mixed solvent with an inert solvent such as dichloromethane or chloroform; or by reacting with ammonia in an alcohol solvent such as methanol or ethanol; or by reacting with an inorganic base such as sodium carbonate or sodium hydrogen carbonate in a mixed solvent of water and a low-molecular-weight alcohol.

The compound having chemical formula 46 can be obtained by reacting dimethyl sulfate in the presence of potasssium carbonate with the compound resulting from the reaction of the

compound having chemical formula 45 with pyridinium dichromate in N,N-dimethylformamide solvent. Dimethyl sulfate may be replaced with a methyl halide such as methyl iodide or diazomethane, whereas it is possible to use an inorganic base like sodium carbonate or an organic base such as DBU or n-butyllithium instead of potassium carbonate. Acetone may be used as a solvent; and it is also possible to mix water with an inert solvent like ethyl ether, petroleum ether, dichloromethane, tetrahydrofuran, or N,N-dimethylformamide.

The compound having chemical formula 48 can be obtained by reacting the compound having chemical formula 47 with tetrabutylammonium fluoride, wherein a reagent that can generate a fluoride, like triethylamine trihydrofluoride or hydrogen fluoride-pyridine, may be used instead of tetrabutylammonium fluoride, or it is preferred to use an organic acid such as p-toluenesulfonic acid or an inorganic acid like hydrochloric acid.

The compound of the present invention has an asymmetric carbon, and therefore the racemic mixture of the aforementioned general formula (I) and R and S stereoisomers are also included in the scope of the present invention. The present invention also includes the pharmaceutically acceptable salts of the compound.

The pharmaceutically acceptable salts of the aforementioned general formula (I) include salts of the acidic or basic group that can be present in the compound of the general formula (I), unless indicated otherwise. For example, the pharmaceutically acceptable salts include sodium, calcium, and potassium salts of the hydroxyl group, whereas other pharmaceutically acceptable salts of the amino group are hydrobromide, sulfate salt, hydrogen sulfate salt, phosphate salt, hydrogen phosphate salt, dihydrogen phosphate salt, acetate, succinate, citrate, tartrate, lactate, mandelate, methanesulfonate (mesylate), and p-toluenesulfonate (tosylate) salt. These salts can be manufactured using manufacturing methods of salts known in the corresponding industry.

The compound of the aforementioned general formula (I) has an asymmetric center, and therefore exists in different mirror-image isomers. All optical isomers and stereoisomers of the compound having general formula (I) and their mixtures are included within the scope of the present invention. The present invention includes application of racemic isomers, more than one mirror image isomer, more than one partial [sic] stereoisomer, or their mixtures. The present invention also includes separation methods or manufacturing methods of isomers that are known in the corresponding industry.

Another objective of the present invention is to provide a pharmacological composition that has selective activity for adenosine A_3 receptors, which contains an effective amount of the compound having the aforementioned general formula (I) and the pharmacutically acceptable carrier, auxiliary material, or diluent.

The aforementioned cancer includes lung cancer, bone cancer, pancreatic cancer, skin cancer, head or neck cancer, melanosis or melanosis bulbi [sic], uterine cancer, ovarian cancer,

rectal cancer, stomach cancer, periproctic cancer, colon cancer, breast cancer, cancerous Fallopian tube tumor, cancerous endometrioma, cancerous cervical tumor, cancerous vaginal tumor, cancerous vulval tumor, Hodgkin's disease, cancer of the esophagus, small intestine cancer, cancer of the endocrine glands, thyroid cancer, parathyroid cancer, adrenal cancer, soft tissue sarcoma, urethral cancer, phallus cancer, prostate cancer, chronic or acute leukemia, lymphocytic lymphoma, bladder cancer, kidney or ureter cancer, cancerous renal tumor, cancerous tumor of the renal pelvis, tumor in central nervous system (CNS), primary CNS lymphoma, myeloma, nerve tumor in brain stem, pituitary gland adenoma, or combinations of two or more of these cancers.

The aforementioned inflammation includes alteriative [transliteration; possibly ulcerative] inflammation, exudative inflammation, purulent inflammation, hemorrhagic inflammation, and hyperplastic inflammation.

In addition, the present invention provides a composition for prevention and treatment of cancer or inflammatory diseases, which composition contains the compound of the aforementioned general formula (I) as an active ingredient and the pharmacutically acceptable carrier.

For example, the compound of the present invention may be dissolved in an oil, propylene glycol, or other solvent that is conventionally used for manufacturing injectable solutions. Appropriate carriers are not specifically limited, but include saline solution, polyethylene glycol, ethanol, vegetable oil, and isopropyl myristate. For topical application, the compound of the present invention may be formulated as an ointment or cream.

The formulation methods and formulating agents are described below, however, they are not limited to these examples.

As a pharmacological formulation of the compound of the present invention, pharmaceutically acceptable salts of the compound may be used, alone or in an appropriate combination, including combinations with other pharmaceutically active ingredients.

The compound of the present invention may be dissolved, suspended, or emulsified in an aqueous solvent of saline and 5% dextrose or in a nonaqueous solvent such as vegetable oil, synthetic fatty acid glyceride, high-molecular-weight fatty acid ester, or propylene glycol to formulate an injectable solution. The formulation of the present invention may include existing additives like disintegrants, isotonic agents, suspending agents, emulsifiers, stabilizers, and preservatives.

An ideal dose of the compound of the present invention depends on the condition and weight of the patient, severity of the disease, drug type, and administration route and period, but may be selected properly by the corresponding professionals. However, it is preferred to administer the compound of the present invention at a daily dose of 0.001-100 mg/kg body

weight, more preferably 0.01-30 mg/kg body weight. The administration may be done once or several times a day. The composition may contain the compound of the present invention at a level of 0.0001-10 wt% of the total amount of the entire composition, more preferably 0.001-1 wt%.

The pharmaceutical composition of the present invention may be administered via various routes to mammals like rats, mice, domestic animals, and humans. It is anticipated that all administration methods can be allowed. For example, oral, rectal, intravenous, intramuscular, hypodermic, intrauterinedural or intracerbroventricular injection may be administered.

Examples and experimental examples of the present invention are described below in detail.

The following experimental examples and test examples are used to illustrate the present invention, but the content of the present invention is not limited to these examples.

Experimental Example 1. Preparation of (2R,3S,4S,5R)-2,3:5,6-di-O-isopropylidene-D-gulono-1,4-lactone compound (1)

20.2 g of D-gulonic γ-lactone (0.114 mol) and 27 g of anhydrous copper(II) sulfate (0.170 mol) were suspended in 0.65 L of dry acetone at ambient temperature, and 1.7 mL of concentrated sulfuric acid were added, which was then stirred for 24 h. The acidity was adjusted to 7 with calcium hydroxide, and then precipitated solids were filtered off. The resulting filtrate was concentrated under reduced pressure to obtain 28.7 g of (2R,3S,4S,5R)-2,3:5,6-di-O-isopropylidene-D-gulono-1,4-lactone compound (yield: 98%) as an oil, which was used immediately for the next reaction.

Experimental Example 2. Preparation of {(4R)-(2,2-dimethyl[1,3]dioxolan-4-yl)}-{(4R,5S)-(5-hydroxymethyl-2,2-dimethyl[1,3]dioxolan-4-yl)}-(S)-methanol compound (2)

25.1 g (0.097 mol) of (2R,3S,4S,5R)-2,3:5,6-di-O-isopropylidene-D-gulono-1,4-lactone compound (1) from the aforementioned Experimental Example 1 were dissolved in 450 mL of ethyl ether to which 7.2 g (0.190 mol) of lithiumaluminum hydride were carefully added in several portions. The reaction solution was stirred at ambient temperature for 10 h, and 7.2 mL of water, 7.2 mL of 15% aqueous sodium hydroxide solution, 22 mL of water, and 19 g of anhydrous magnesium sulfate were sequentially added at 0°C. The resulting solution was filtered and the filtrate was concentrated under reduced pressure. The concentrate was purified by silica gel column chromatography using the eluant hexane:ethyl acetate = 1:1. 24.1 g (yield: 95%) of {(4R)-(2,2-dimethyl[1,3]dioxolan-4-yl)}-{(4R,5S)-(5-hydroxymethyl-2,2-dimethyl[1,3]dioxolan-4-yl)}-(S)-methanol were obtained as an oil.

^1H-NMR($CDCl_3$) δ : 1.29 (d, 6H), 1.36 (s, 3H), 1.43 (s, 3H), 3.26 (s, 1H), 3.28 (s, 1H), 3.36-3.79 (m, 4H), 3.96-4.03 (m, 2H), 4.12-4.22 (m, 2H)

Experimental Example 3. Preparation of methanesulfonic acid {(4R)-(2,2-dimethyl[1,3]dioxolan-4-yl)}-{(4S,5S)-(5-methanesulfonyloxymethyl-2,2-dimethyl[1,3]dioxolan-4-yl)}-(S) methyl ester compound (3)

4.1 g (0.016 mol) of {(4R)-(2,2-dimethyl[1,3]dioxolan-4-yl)}-{(4R,5S)-(5-hydroxymethyl-2,2-dimethyl[1,3]dioxolan-4-yl)}-(S)-methanol from the aforementioned Experimental Example 2 were dissolved in a mixed solvent of 1.0 L dichloromethane and 25 mL pyridine. After cooling the mixture to 0°C, 18 mL (0.236 mol) of methanesulfonyl chloride were added and the resulting solution was stirred for 5 h at 0°C. Saturated sodium bicarbonate solution was added and the compound was extracted with chloroform. The extract was dried, filtered, and concentrated under reduced pressure. The concentrate was purified by silica gel column chromatography using the eluant hexane:ethyl acetate = 2:1 to obtain 6.4 g (yield: 98%) of methanesulfonic acid {(4R)-(2,2-dimethyl[1,3]dioxolan-4-yl)}-{(4S,5S)-(5-methanesulfonyloxymethyl-2,2-dimethyl[1,3]dioxolan-4-yl)}-(S) methyl ester as an oil.

^1H-NMR($CDCl_3$) δ : 1.37 (s, 3H), 1.39 (s, 3H), 1.46 (s, 3H), 1.52(s, 3H), 3.10 (s, 3H), 3.18 (s, 3H), 3.92-4.08 (m, 1H), 4.08-4.20 (m, 2H), 4.39-4.49 (m, 4H), 4.81-4.87 (m, 1H)

Experimental Example 4. Preparation of (3aS,4R,6aR)-4-((4R)-2,2-dimethyl[1,3]dioxolan-4-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole compound (4)

10.1 g (0.024 mol) of methanesulfonic acid {(4R)-(2,2-dimethyl[1,3]dioxolan-4-yl)}-{(4S,5S)-(5-methanesulfonyloxymethyl-2,2-dimethyl[1,3]dioxolan-4-yl)}-(S) methyl ester from the aforementioned Experimental Example 3 were dissolved in 260 mL of N,N-dimethylformamide, and 8.9 g (0.036 mol) of sodium sulfide were added. The reaction mixture was reacted at 100°C for 3 h. The reaction mixture was concentrated under reduced pressure, and water was added to the concentrate, which was then extracted with ethyl acetate. The organic layer was dried and filtered, and the filtrate was concentrated under reduced pressure. The concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl

acetate = 5:1) to obtain 5.9 g (yield: 94%) of (3aS,4R,6aR)-4-((4R)- 2,2-dimethyl[1,3]dioxolan-4-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole as an oil.

^{1}H-NMR($CDCl_3$) δ : 1.33 (d, 6H), 1.44 (s, 3H), 1.51 (s, 3H), 2.86 (d, 1H), 3.09 (m, 1H), 3.22 (s, 1H), 3.76 (dd, 1H), 3.98 (m, 1H), 4.15 (dd, 1H), 4.93 (d, 2H)

Experimental Example 5. Preparation of (1R)-1-(3aS,4R,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl)-ethane-1,2-diol compound (5)

5.4 g (0.020 mol) of (3aS,4R,6aR)-4-((4R)-(2,2-dimethyl[1,3]dioxolan-4-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole obtained from the aforementioned Experimental Example 4 were added to 150 mL of 60% aqueous acetic acid solution, which was stirred at ambient temperature for 50 h. The reaction mixture was concentrated under reduced pressure, and the concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 1:1) to obtain 3.4 g (yield: 75%) of (1R)-1-((3aS,4R,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl)-ethane-1,2-diol as an oil.

^{1}H-NMR($CDCl_3$) δ :1.34 (s, 3H), 1.53 (s, 3H), 2.90 (dd, 1H), 3.09 (dd, 1H), 3.27 (dd, 1H), 3.58-3.65 (m, 2H), 3.69 (dd, 1H), 3.79 (dd, 1H), 4.15 (dd, 1H), 4.93 (m, 2H)

Experimental Example 6. Preparation of (3aS,4S,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole-4-carbaldehyde compound (6)

2.5 g (11.2 mmol) of (1R)-1-((3aS,4R,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl)-ethane-1,2-diol from the aforementioned Experimental Example 5 were dissolved in ethyl acetate to which lead tetraacetate were added at 0°C, and the reaction mixture was stirred for 10 min. The reaction mixture was filtered. Ethyl acetate was added to the filtrate, which was washed with saturated sodium bicarbonate, dried, and filtered. The filtrate was concentrated under reduced pressure, and the concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 5:1) to obtain 2.1 g (yield: 98%) of (3aS,4S,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole-4-carbaldehyde as an oil.

^{1}H-NMR($CDCl_3$) δ :1.33 (s, 3H), 1.52 (s, 3H), 2.61(dd, 1H), 2.87 (dd, 1H), 3.92 (s, 1H), 4.92 (t, 1H), 5.10 (d, 1H), 9.43 (s, 1H)

Experimental Example 7. Synthesis of (3aS,4S,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole-4-carboxylic acid methyl ester compound (7)

2.8 g (0.015 mol) of (3aS,4S,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole-4-carbaldehyde from the aforementioned Experimental Example 6 were dissolved in 200 mL of anhydrous N,N-dimethylformamide to which 26.7 g (0.070 mol) of pyridinium dichromate was added. The reaction mixture was stirred at ambient temperature for 20 h. After 0.3 L of water were added, it was extracted with 2.5 L of ethyl acetate (0.5 L x 5). The extract was dried, filtered, and concentrated under reduced pressure to obtain the concentrate which was (3aS,4S,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole-4-carboxylic acid. The concentrate was dissolved in 65 mL of acetone to which 2 mL (21.14 mmol) of dimethyl sulfate and 1.2 g (8.68 mmol) of potassium carbonate were added. The resulting mixture was stirred for 2 h. Acetone was distilled off under reduced pressure, and the residue was dissolved in ethyl acetate, followed by washing with water and saturated saline solution. The organic layer was dried and filtered, and the filtrate was concentrated under reduced pressure. The concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 3:1) mixture to obtain 2.5 g (yield: 78%) of (3aS,4S,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole-4-carboxylic acid methyl ester as an oil.

^{1}H-NMR($CDCl_3$) δ : 1.33 (s, 3H), 1.52 (s, 3H), 2.92 (d, 1H), 3.19 (dd, 1H), 3.72 (s, 3H), 3.80 (s, 1H), 4.99 (m, 2H)

Experimental Example 8. Synthesis of (3aS,4R,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole-4-carboxylic acid methyl amide compound (8)

1.1 g (0.005 mol) of (3aS,4R,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole-4-carboxylic acid methyl ester compound (7) from the aforementioned Experimental Example 7 were dissolved in 40 mL (0.080 mol) of 2N methylamine solution in tetrahydrofuran, which was stirred at 50°C for 24 h in a sealed container. After concentration under reduced pressure, the obtained concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 2:1) to obtain 953 mg (yield: 87%) of (3aS,4S,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole-4-carboxylic acid methyl amide as an oil.

^{1}H-NMR($CDCl_3$) δ : 1.29 (s, 3H), 1.48 (s, 3H), 2.78 (d, 3H), 2.91 (d, 1H), 3.04 (dd, 1H), 3.72 (s, 1H), 4.91 (t, 1H), 5.21 (d, 1H), 6.65 (br s, 1H).

Experimental Example 9. Synthesis of (3aS,4S,5R,6aR)- and (3aS,4S,5S,6aR)-2,2-dimethyl-5-oxotetrahydro-5λ^4-thieno[3,4-d][1,3]dioxole-4-carboxylic acid methyl amide compound (9)

120 mg (0.55 mmol) of (3aS,4R,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole-4-carboxylic acid methyl amide from the aforementioned Experimental Example 8 were dissolved in 15 mL of dichloromethane. After cooling to -78°C, 110 mg (0.55 mmol, 80% reagent) of m-chloroperbenzoic acid dissolved in 5 mL of dichloromethane were added slowly. The reaction mixture was stirred at -78°C for 45 min, and saturated sodium bicarbonate solution was added, followed by extraction with dichloromethane. The organic layer was washed with saturated saline solution, dried, filtered, and concentrated under reduced pressure. The resulting concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 1:1.2) to obtain 125 mg (yield: 97%) of (3aS,4S,5R,6aR)- and (3aS,4S,5S,6aR)-2,2-dimethyl-5-oxotetrahydro-5λ^4-thieno[3,4-d][1,3]dioxole-4-carboxylic acid methyl amide as an oil.

^{1}H-NMR($CDCl_3$) δ : 1.23 (s, 3H), 1.37 (s, 3H), 2.80 (d, 3H), 3.21 (dd, 1H), 3.61 (d, 1H), 4.30 (s, 1H), 5.06 (m, 2H), 7.12 (br s, 1H)

Experimental Example 10. Synthesis of (3aS,4R,6aR)-4-(2,6-dichloropurin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole-4-carboxylic acid methyl amide compound (10)

102 mg (0.54 mmol) of 2,6-dichloropurine and 20 mg (0.15 mmol) of ammonium sulfate were suspended in 10 mL of anhydrous hexamethyldisilazane and stirred under reflux for 6 h. The reaction mixture was concentrated under reduced pressure and dry conditions, and the concentrate was dissolved in 8 mL of dichloroethane. 110 mg (0.47 mmol) of (3aS,4S,5R,6aR)- and (3aS,4S,5S,6aR)-2,2-dimethyl-5-oxotetrahydro-5λ^4-thieno[3,4-d][1,3]dioxole-4-carboxylic acid methyl amide dissolved in 5 mL of dichloroethane were added, followed by cooling to -10°C. After adding 0.10 mL (0.54 mmol) of trimethylsilyltrifluoromethanesulfonate, the reaction mixture was stirred at -10°C for 20 min, and then stirred under reflux for 4 h. Saturated sodium bicarbonate solution was added and the mixture stirred for 15 min, followed by extraction with

dichloromethane. The extract was washed with saturated saline solution, dried, filtered, and concentrated under reduced pressure. The concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 1.5:1) to obtain 103 mg (yield: 54%) of (3aS,4R, 6aR)-4-(2,6-dichloropurin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole-4-carboxylic acid methyl amide as an oil.

^{1}H-NMR($CDCl_3$) δ : 1.42 (s, 3H), 1.61 (s, 3H), 2.83 (d, 3H), 3.07 (m, 2H), 5.11 (m, 1H), 6.09 (d, 1H), 7.24 (br s, 1H), 8.52 (s, 1H)

UV (methanol): λ_{max} 270 nm (pH 7)

Experimental Example 11. Synthesis of (3aS,4R,6aR)-4-[2-chloro-6-(3-iodobenzylamino)purin-9-yl)]-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole-4-carboxylic acid methyl amide compound (11)

65 mg (0.16 mmol) of (3aS,4R,6aR)-4-(2,6-dichloropurin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole-4-carboxylic acid methyl amide from the aforementioned Experimental Example 10, 55 mg (0.20 mmol) of 3-iodobenzylamine hydrochloride, and 0.065 mL (0.46 mmol) of triethylamine were added to ethanol and stirred at ambient temperature for 3 days. The reaction mixture was distilled under reduced pressure to obtain a concentrate. The concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 1:1) to obtain 87 mg (yield: 90%) of (3aS,4R,6aR)-4-[2-chloro-6-(3-iodobenzylamino)purin-9-yl)]-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole-4-carboxylic acid methyl amide as a white foam.

^{1}H-NMR($CDCl_3$) δ : 1.43 (s, 3H), 1.61 (s, 3H), 2.88 (d, 3H), 3.07 (d, 2H), 4.73 (br d, 2H), 5.09 (m, 1H), 6.11 (d, 1H), 6.49 (br s, 1H), 7.07 (t, 1H), 7.32 (d, 1H), 7.60 (d, 1H), 7.72 (s, 1H), 8.16 (s, 1H)

UV (methanol): λ_{max} 271 nm (pH 7)

Experimental Example 12. Synthesis of (2R,3S,4R)-2-[2-chloro-6-(3-iodobenzylamino)purin-9-yl)-3,4-dihydroxytetrahydrothiophene-2-carboxylic acid methyl amide compound (12)

4 mL of 80% aqueous acetic acid solution were added to 42 mg (0.07 mmol) of (3aS,4R,6aR)-4-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole-4-carboxylic acid methyl amide from the aforementioned Experimental Example 11, which was then stirred at 55°C for 12 h. The reaction mixture was distilled under reduced pressure, and then the pH of the concentrate was adjusted to neutral by adding a saturated ammonia methanol solution. The concentrate was purified by silica gel column chromatography using the eluant (dichloromethane and methanol = 7:1) to obtain 20 mg (yield: 52%) of (2R,3S,4R)-2-[2-chloro-6-(3-iodobenzylamino)purin-9-yl)-3,4-dihydroxytetrahydrothiophene-2-carboxylic acid methyl amide as white solids (see Table 1).

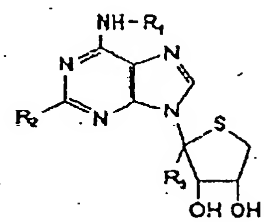
Melting point: 205-207°C

$[\alpha]^{26}_{D}$: -24.5 (c 0.06, methanol)

υ_{max} (KBr)/cm^{-1} : 1066, 1375, 1455, 2924

^{1}H-NMR(DMSO-d_6) δ : 2.63 (d, 3H), 2.99 (m, 1H), 3.15 (m, 1H), 4.21 (br s, 1H), 4.73 (br d, 2H), 5.03 (br s, 1H), 5.68 (d, 1H), 6.45 (d, 1H), 7.12 (t, 1H), 7.33 (d, 1H), 7.58 (d, 1H), 7.73 (s, 1H), 8.13 (s, 1H), 8.25 (br s, 1H), 8.73 (br s, 1H)

UV (methanol): λ_{max} 271 nm (pH 7)



(II)

Table 1

Compound group	Compound	R_1	R_2	R_3	R_3	Spectral data
II	12	3-Iodobenzyl	Chlorine	Methyl-amino-carbonyl	Hydrogen	^{1}H-NMR(DMSO-d_6) δ : 2.63 (d, 3H), 2.99 (m, 1H), 3.15 (m, 1H), 4.21 (br s, 1H), 4.73 (br d, 2H), 5.03 (br s, 1H), 5.68 (d, 1H), 6.45 (d, 1H), 7.12 (t, 1H), 7.33 (d, 1H), 7.58 (d, 1H), 7.73 (s, 1H), 8.13 (s, 1H), 8.25 (br s, 1H), 8.73 (br s, 1H)

Experimental Example 13. Synthesis of (3aS,4R,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl)-methanol compound (13)

5.6 g (30.0 mmol) of (3aS,4S,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxole-4-carbaldehyde were dissolved in 70 mL of methanol to which 1.3 g (33.6 mmol) of sodium borohydride were added in several portions at 0°C. The reaction mixture was stirred for 30 min at ambient temperature and neutralized with acetic acid. After distillation under reduced pressure, saturated saline solution was added and the product was extracted with ethyl acetate. The extract was dried and filtered. The filtrate was distilled under reduced pressure to obtain a concentrate. The concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 2:1) to obtain 5.5 g (yield; 98%) of (3aS,4R,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl)-methanol as white foam.

^{1}H-NMR($CDCl_3$) δ : 1.33 (s, 3H), 1.53 (s, 3H), 2.41 (br s, 1H), 2.89 (dd, 1H), 3.09 (dd, 1H), 3.44 (dt, 1H), 3.59 (d, 2H), 4.71 (dd, 1H), 4.91 (dt, 1H)

Experimental Example 14. Synthesis of benzoic acid (3aS,4R,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester compound (14)

2.1 g (11.1 mmol) of (3aS,4R,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl)-methanol from the aforementioned Experimental Example 13 were dissolved in 20 mL of pyridine to which 1.9 g (13.4 mmol) of benzoyl chloride were added at 0°C. The reaction mixture was stirred at ambient temperature for 6 h, and then 2 mL of methanol were added. After distillation of the reaction mixture under reduced pressure, 50 mL of diethyl ether were added and the solids formed were filtered. The filtrate was distilled under reduced pressure to obtain the concentrate. The concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 4:1) to obtain 3.2 g (yield: 99%) of benzoic acid (3aS,4R,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester as a colorless oil.

^{1}H-NMR($CDCl_3$) δ : 1.26 (s, 3H), 1.47 (s, 3H), 2.87 (dd, 1H), 3.09 (dd, 1H), 3.44 (dt, 1H), 4.28 (m, 2H), 4.72 (dd, 1H), 4.91 (dt, 1H), 7.35-7.99 (m, 5H)

Experimental Example 15. Synthesis of benzoic acid (3aS,4R,5R,6aR)-(3aS,4R,5S,6aR)-2,2-dimethyl-5-oxotetrahydro-5λ^4-thieno[3,4-d][1,3]dioxol-4-yl methyl ester compound (15)

1.4 g (4.6 mmol) of benzoic acid (3aS,4R,6aR)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester from the aforementioned Experimental Example 14 were dissolved in 30 mL of dichloromethane. After cooling the reaction mixture to -78°C, 1.0 g (4.6 mmol) of m-chloroperbenzoic acid solution in 15 mL of dichloromethane was added dropwise, and the reaction mixture was stirred at -78°C for 45 min. Saturated sodium bicarbonate solution was added and the product was extracted with dichloromethane. The organic layer was washed with saturated saline solution, dried, and filtered. The filtrate was distilled under reduced pressure, and the concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 2:1) to obtain 3.2 g (yield: 99%) of benzoic acid (3aS,4R,5R,6aR)-(3aS,4R,5S,6aR)-2,2-dimethyl-5-oxotetrahydro-5λ^4-thieno[3,4-d][1,3]dioxol-4-yl methyl ester as a solid.

^{1}H-NMR($CDCl_3$) δ : 1.33 (s, 3H), 1.49 (s, 3H), 3.21 (dd, 1H), 3.37 (dd, 1H), 3.47 (m, 1H), 4.72 (dd, 1H), 4.87 (dd, 1H), 5.02 (t, 1H), 5.24 (m, 1H), 7.41-8.03 (m, 5H).

Experimental Example 16. Synthesis of benzoic acid (3aS,4R,6R,6aR)-(3aS,4R,6S,6aR)-6-acetoxy-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester compound (16)

3.5 g (11.3 mmol) of benzoic acid (3aS,4R,5R,6aR)-(3aS,4R,5S,6aR)-2,2-dimethyl-5-oxotetrahydro-5λ^4-thieno[3,4-d][1,3]dioxol-4-yl methyl ester from the aforementioned Experimental Example 15 were dissolved in 90 mL of acetic anhydride, followed by stirring at 100°C for 6 h. After the reaction mixture was concentrated under reduced pressure, water was added and the product was extracted with ethyl acetate. The organic layer was washed with saturated sodium bicarbonate and then saturated saline solution. The organic layer was dried, filtered, and distilled under reduced pressure to obtain the concentrate. The concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 3:1) to obtain 2.5 g (yield: 62%) of benzoic acid (3aS,4R,6R,6aR)-(3aS,4R,6S,6aR)-6-acetoxy-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester.

^{1}H-NMR($CDCl_3$) δ : 1.30 (s, 3H), 1.50 (s, 3H), 2.03 (s, 3H), 3.76 (dd, 1H), 4.39 (m, 2H), 4.94 (d, 1H), 4.98 (d, 1H), 6.06 (s, 1H), 7.42-8.06 (m, 5H)

Experimental Example 17. Synthesis of benzoic acid (3aS,4R,6R,6aR)- and (3aS,4R,6S,6aR)-6-(2,6-dichloropurin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester compound (17)

415 mg (2.2 mmol) of 2,6-dichloropurine and a catalytic amount of ammonium sulfate were suspended in 80 mL of anhydrous hexamethyldisilazane and stirred under reflux for 6 h. The reaction mixture was concentrated under reduced pressure and dry conditions, and the concentrate was dissolved in 15 mL of dichloroethane. 668 mg (1.9 mmol) of benzoic acid (3aS,4R,6R,6aR)- and (3aS,4R,6S,6aR)-6-acetoxy-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester dissolved in 20 mL of dichloroethane were added, followed by cooling to -10°C. After adding 0.42 mL (2.18 mmol) of trimethylsilyl trifluoromethanesulfonate, the reaction mixture was stirred at -10°C for 20 min, and then stirred under reflux for 4 h. Saturated sodium bicarbonate solution was added and the mixture was stirred for 15 min and then extracted with dichloromethane. The extract was washed with saturated saline solution, dried, filtered, and concentrated under reduced pressure. The concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 2:1 by volume) to obtain 409 mg (yield: 44%) of benzoic acid (3aS,4R,6R,6aR)-6-(2,6-dichloropurin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester and 102 mg (yield: 11%) of benzoic acid (3aS,4R,6S,6aR)-6-(2,6-dichloropurin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester as white foam, respectively.

β-anomer (3aS,4R,6R,6aR)

^{1}H-NMR($CDCl_3$) δ : 1.39 (s, 3H), 1.64 (s, 3H), 4.11 (dt, 1H), 4.57 (dd, 1H), 4.75 (dd, 1H), 5.19 (dd, 1H), 5.40 (dd, 1H), 6.10 (d, 1H), 7.37-7.97 (m, 5H), 8.38 (s, 1H)

UV (methanol): λ_{max} 269 nm (pH 7)

α-anomer (3aS,4R,6S,6aR)

^{1}H-NMR($CDCl_3$) δ : 1.37 (s, 3H), 1.64 (s, 3H), 4.08 (m, 1H), 4.51 (dd, 1H), 4.62 (dd, 1H), 5.01 (m, 1H), 5.11 (m, 1H), 6.50 (d, 1H), 7.37-7.99 (m, 5H), 8.87 (s, 1H)

UV (methanol): λ_{max} 279 nm (pH 7)

Experimental Example 18. Synthesis of benzoic acid (3aS,4R,6R,6aR)-6-(2-chloro-6-(3-iodobenzylamino)purin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester compound (18)

1.5 mL of ethanol were added to 254 mg (0.53 mmol) of benzoic acid (3aS,4R,6R,6aR)-6-(2,6-dichloropurin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester compound from the aforementioned Experimental Example 17, 200 mg (0.68 mmol) of 3-iodobenzylamine hydrochloride, and 0.22 mL (1.53 mmol) of triethylamine, which was stirred at ambient temperature for 3 days. The reaction mixture was distilled under reduced pressure, and the concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 1:1) to obtain 322 mg (yield: 90%) of benzoic acid (3aS,4R,6R,6aR)-6-(2-chloro-6-(3-iodobenzylamino)purin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester as white foam.

^{1}H-NMR($CDCl_3$) δ : 1.37 (s, 3H), 1.63 (s, 3H), 4.11 (dt, 1H), 4.57 (dd, 1H), 4.76 (m, 3H), 5.22 (dd, 1H), 5.38 (dd, 1H), 6.01 (d, 1H), 6.28 (br, 1H), 7.04-8.00 (m, 9H), 8.02 (s, 1H)

UV (methanol): λ_{max} 272 nm (pH 7)

Experimental Example 19. Synthesis of benzoic acid (3aS,4R,6S,6aR)-6-(2-chloro-6-(3-iodobenzylamino)purin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester compound (19)

1.5 mL of ethanol were added to 80 mg (0.17 mmol) of benzoic acid (3aS,4R,6S,6aR)-6-(2,6-dichloropurin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester, 60 mg (0.22 mmol) of 3-iodobenzylamine hydrochloride, and 0.07 mL (0.49 mmol) of triethylamine, which was stirred at ambient temperature for 3 days. The reaction mixture was distilled under reduced pressure, and the concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 1:1) to obtain 102 mg (yield: 90%) of benzoic acid (3aS,4R,6S,6aR)-6-(2-chloro-6-(3-iodobenzylamino)purin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester as white foam.

^{1}H-NMR($CDCl_3$) δ : 1.23 (s, 3H), 1.28 (s, 3H), 3.89 (t, 1H), 3.94 (dd, 1H),

4.08 (dd, 1H), 4.65 (d, 2H), 5.01 (m 2H), 5.81 (d, 1H), 6.91-7.75 (m, 9H), 8.83 (s, 1H)

UV (methanol): λ_{max} 282 nm (pH 7)

Experimental Example 20. Synthesis of (2R,3R, 4S,5R)-2-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-5-hydroxymethyltetrahydrothiophene-3,4-diol compound (20)

6 mL of 80% aqueous acetic acid solution were added to 122 mg (0.18 mmol) of (3aS,4R,6R, 6aR)-6-[2-chloro-6-(3-iodobenzylamino)purin-9-yl)]-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester, which was then stirred at 55°C for 12 h. The reaction mixture was distilled under reduced pressure, and then the pH of the concentrate was adjusted to neutral by adding saturated ammonia methanol solution. The concentrate was purified by silica gel column chromatography using the eluant (dichloromethane and methanol = 15:1) to obtain (2R,3S,4R,5R)-5-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-3,4-dihydroxytetrahydrothiophen-2-yl methyl ester as a white foam. The aforementioned (2R,3S,4R,5R)-5-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-3,4-dihydroxytetrahydrothiophen-2-yl methyl ester was dissolved in 8 mL of methanol to which 30 mg (0.58 mmol) of sodium methoxide were added. The reaction mixture was stirred at ambient temperature for 4 h and the pH of the solution was adjusted to neutral with acetic acid, followed by distillation under reduced pressure. The concentrate was purified by silica gel column chromatography using the eluant (dichloromethane:methanol = 6:1) to obtain 51 mg (yield: 53%) of (2R,3R,4S,5R)-2-[2-chloro-6-(3-iodobenzylamino)purin-9-yl)-5-hydroxymethyltetrahydrothiophene-3,4-diol as white solids (see Table 3).

Melting point: 112-114°C

$[\alpha]^{26}_{D}$: -28.3 (c 0.1, methanol)

υ_{max} (KBr)/cm^{-1} : 1316, 1622, 3400

^{1}H-NMR(DMSO-d_6) δ : 3.29 (m, 1H), 3.62 (m, 1H), 3.78 (m, 1H), 4.20 (d, 1H), 4.61 (m, 3H), 5.17 (t, 1H), 5.34 (d, 1H), 5.58 (d, 1H), 5.77 (d, 1H), 7.13 (t, 1H), 7.34 (d, 1H), 7.59 (d, 1H), 7.74 (s, 1H), 8.53 (s, 1H), 8.92 (br t, 1H)

^{13}C-NMR(DMSO-d_6):δ 42.2, 53.1, 61.1, 62.8, 72.8, 76.7, 126.5, 130.2, 135.3, 135.7, 140.4

UV (methanol): λ_{max} 273 nm (pH 7)

Experimental Example 21. Synthesis of (2S,3R, 4S,5R)-2-[2-chloro-6-(3-iodobenzylamino)purin-9-yl)-5-hydroxymethyltetrahydrothiophene-3,4-diol compound (21)

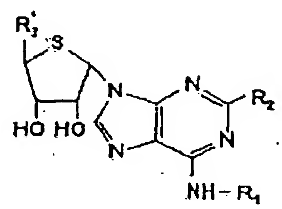
3 mL of 80% aqueous acetic acid solution were added to 50 mg (0.07 mmol) of (3aS,4R,6S,6aR)-6-[2-chloro-6-(3-iodobenzylamino)purin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester, which was then stirred at 55°C for 12 h. The reaction mixture was distilled under reduced pressure, and then the pH of the concentrate was adjusted to neutral by adding saturated ammonia methanol solution. The concentrate was purified by silica gel column chromatography using the eluant (dichloromethane and methanol = 15:1) to obtain (2R,3S,4R,5S)-(5-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-3,4-dihydroxytetrahydrothiophen-2-yl methyl ester as white foam. The aforementioned (2R,3S,4R,5S)-(5-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-3,4-dihydroxytetrahydrothiophen-2-yl methyl ester was dissolved in 4 mL of methanol to which 15 mg (0.29 mmol) of sodium methoxide were added. The reaction mixture was stirred at ambient temperature for 4 h and the pH of the solution was adjusted to neutral with acetic acid, followed by distillation under reduced pressure. The concentrate was purified by silica gel column chromatography using the eluant (dichloromethane:methanol = 6:1) to obtain 21 mg (yield: 54%) of (2S,3R,4S,5R)-2-[2-chloro-6-(3-iodobenzylamino)purin-9-yl)-5-hydroxymethyltetrahydrothiophene-3,4-diol as white solids (see Table 2).

Melting point: 123-124°C

υ_{max} (KBr)/cm^{-1} : 1557, 1608, 3429

^{1}H-NMR(DMSO-d_6) δ : 3.43 (br d, 1H), 3.56 (dd, 1H), 3.71 (dd, 1H), 4.06 (dd, 1H), 4.27 (dd, 1H), 4.57 (dd, 1H), 4.69 (dd, 1H), 5.42 (d, 1H), 5.57 (t, 1H), 5.58 (d, 1H), 5.95 (d, 1H), 7.09 (t, 1H), 7.33 (d, 1H), 7.54 (d, 1H), 7.73 (s, 1H), 8.20 (br s, 1H), 8.59 (s, 1H)

UV (methanol): λ_{max} 283 nm (pH 7)



(IV)

(IV)

Table 2

Compound group	Compound	R_1	R_2	R_3	R_3	Spectral data
IV	21	3-Iodobenzyl	Chlorine	Hydrogen	Hydroxy-methyl	^{1}H-NMR(DMSO-d_6) δ : 3.43 (br d, 1H), 3.56 (dd, 1H), 3.71 (dd, 1H), 4.06 (dd, 1H), 4.27 (dd, 1H), 4.57 (dd, 1H), 4.69 (dd, 1H), 5.42 (d, 1H), 5.57 (t, 1H), 5.58 (d, 1H), 5.95 (d, 1H), 7.09 (t, 1H), 7.33 (d, 1H), 7.54 (d, 1H), 7.73 (s, 1H), 8.20 (br s, 1H), 8.59 (s, 1H)

Experimental Example 22. Synthesis of benzoic acid (3aS,4R,6R,6aR)-6-[2-chloro-6-methylaminopurin-9-yl)-2,2-dimethyltetrahydrothieno-[3,4-d][1,3]dioxol-4-yl methyl ester compound (22)

454 mg (0.94 mmol) of benzoic acid (3aS,4R,6R,6aR)-6-[2,6-dichloropurin-9-yl)-2,2-dimethyltetrahydrothieno-[3,4-d][1,3]dioxol-4-yl methyl ester were dissolved in 20 mL (40 mmol) of 2N methylamine-tetrahydrofuran solution, which was stirred at ambient temperature in a sealed container for 24 h. The reaction mixture was concentrated under reduced pressure, and the concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 1:1) to obtain 383 mg (yield: 85%) benzoic acid (3aS,4R,6R,6aR)-6-[2-

chloro-6-methylaminopurin-9-yl)-2,2-dimethyltetrahydrothieno-[3,4-d][1,3]dioxol-4-yl methyl ester as white foam.

^{1}H-NMR($CDCl_3$) δ : 1.37 (s, 3H), 1.63 (s, 3H), 3.02 (d, 3H), 4.01 (dt, 1H), 4.57 (dd, 1H), 4.75 (dd, 1H), 5.23 (dd, 1H), 5.38 (dd, 1H), 6.01 (d, 1H), 6.28 (br s, 1H), 7.40-8.00 (m, 5H), 8.02 (s, 1H)

UV (methanol): λ_{max} 271 nm (pH 7)

Experimental Example 23. Synthesis of (2R,3R,4S,5R)-2-(2-chloro-6-methylaminopurin-9-yl)-5-hydroxymethyltetrahydrothiophene-3,4-diol compound (23)

5 mL of 80% aqueous acetic acid solution were added to 105 mg (0.22 mmol) of benzoic acid (3aS,4R,6R,6aR)-6-(2-chloro-6-methylaminopurin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester, which was then stirred at 55°C for 12 h. The reaction mixture was distilled under reduced pressure, and then the pH of the concentrate was adjusted to neutral by adding a saturated ammonia methanol solution. The concentrate was purified by silica gel column chromatography using the eluant (dichloromethane and methanol = 15:1) to obtain benzoic acid (2R,3S,4R,5R)-5-[2-chloro-6-methylaminopurin-9-yl]-3,4-dihydroxytetrahydrothiophen-2-yl methyl ester as white foam. The aforementioned (benzoic acid (2R,3S, 4R,5R)-5-[2-chloro-6-methylaminopurin-9-yl]-3,4-dihydroxytetrahydrothiophen-2-yl methyl ester was dissolved in 5 mL of methanol to which 15 mg (0.29 mmol) of sodium methoxide were added. The reaction mixture was stirred at ambient temperature for 4 h and the pH of the solution was adjusted to neutral with acetic acid, which was then distilled under reduced pressure. The concentrate was purified by silica gel column chromatography using the eluant (dichloromethane:methanol = 6:1) to obtain 38 mg (yield: 52%) of ((2R,3R,4S,5R)-2-(2-chloro-6-methylaminopurin-9-yl)-5-hydroxymethyltetrahydrothiophene-3,4-diol as white solids (see Table 3).

Melting point: 239-241°C

$[\alpha]^{24}_D$: -30.6 (c 0.1 methanol)

υ_{max} (KBr)/cm^{-1}: 1312, 1626, 3419

^1H-NMR(DMSO-d_6) δ : 2.92 (d, 3H), 3.30 (br s, 1H), 3.62 (m, 1H), 3.79 (m, 1H), 4.20 (dd, 1H), 4.61 (m, 1H), 5.16 (t, 1H), 5.34 (d, 1H), 5.58 (d, 1H), 5.77 (d, 1H), 8.26 (br s, 1H), 8.47 (s, 1H)

^{13}C-NMR(DMSO-d_6): δ 27.5, 53.7, 61.7, 63.5, 73.5, 77.4, 118.9, 140.5, 150.0, 153.6, 155.9

UV (methanol): λ_{max} 271 nm (pH 7)

Experimental Example 24. Synthesis of benzoic acid (3aS,4R,6R,6aR)-6-[6-amino-2-chloro-purin-9-yl)-2,2-dimethyltetrahydrothieno-[3,4-d][1,3]dioxol-4-yl methyl ester compound (24)

454 mg (0.94 mmol) of benzoic acid (3aS,4R,6R,6aR)-6-[2,6-dichloropurin-9-yl)-2,2-dimethyltetrahydrothieno-[3,4-d][1,3]dioxol-4-yl methyl ester were dissolved in 25 mL of saturated ammonia ethanol solution, which was transferred into a sealed container and heated at 50°C for 12 h. The reaction mixture was concentrated under reduced pressure, and the concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 1:1) to obtain 384 mg (yield: 88%) benzoic acid (3aS,4R,6R,6aR)-6-[6-amino-2-chloro-purin-9-yl)-2,2-dimethyltetrahydrothieno-[3,4-d][1,3]dioxol-4-yl methyl ester as a white foam.

^1H-NMR($CDCl_3$) δ : 1.37 (s, 3H), 1.63 (s, 3H), 4.01 (dt, 1H), 4.57 (dd, 1H), 4.77 (dd, 1H), 5.23 (dd, 1H), 5.38 (dd, 1H), 6.02 (d, 1H), 6.29 (br s, 2H), 7.40~8.00 (m, 5H), 8.02 (s, 1H)

UV (methanol): λ_{max} 264 nm (pH 7)

Experimental Example 25. Synthesis of (2R,3R, 4S,5R)-2-(2-chloro-6-methylaminopurin-9-yl)-5-hydroxymethyltetrahydrothiophene-3,4-diol compound (25)

5 mL of 80% aqueous acetic acid solution were added to 102 mg (0.22 mmol) of benzoic acid (3aS,4R,6R,6aR)-6-(6-amino-2-chloro-purin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester, which was then stirred at 55°C for 12 h. The reaction mixture was distilled under reduced pressure, and then the pH of the concentrate was adjusted to neutral by adding saturated ammonia methanol solution. The concentrate was purified by silica gel column chromatography using the eluant (dichloromethane and methanol = 15:1) to obtain benzoic acid (2R,3S,4R,5R)-5-[6-amino-2-chloro-purin-9-yl]-3,4-dihydroxytetrahydrothiophen-4-yl methyl ester as white foam. The aforementioned benzoic acid (2R,3S,4R,5R)-5-[6-amino-2-chloro-purin-9-yl]-3,4-dihydroxytetrahydrothiophen-4-yl methyl ester was dissolved in 5 mL of methanol to which 15 mg (0.29 mmol) of sodium methoxide were added. The reaction mixture was stirred at ambient temperature for 4 h and the pH of the solution was adjusted to neutral with acetic acid, followed by distillation under reduced pressure. The concentrate was purified by silica gel column chromatography using the eluant (dichloromethane:methanol = 6:1) to obtain 37 mg (yield: 53%) of (2R,3R,4S,5R)-2-(2-chloro-6-methylaminopurin-9-yl)-5-hydroxymethyltetrahydrothiophene-3,4-diol as white solids (see Table 3).

Melting point: 220-222°C

$[\alpha]^{24}_D$: -24.6 (c 0.7 methanol)

υ_{max} (KBr)/cm^{-1} : 1204, 1647, 3420

^{1}H-NMR(DMSO-d_6) δ : 3.30 (br s, 1H), 3.62 (m, 1H), 3.79 (m, 1H), 4.20 (dd, 1H), 4.61 (dd, 1H), 5.16 (t, 1H), 5.34 (d, 1H), 5.58 (d, 1H), 5.77 (d, 1H), 7.81 (br s, 2H), 8.48 (s, 1H)

^{13}C-NMR(DMSO-d_6): δ 53.3, 61.2, 63.1, 73.1, 76.9, 118.0, 140.4, 150.7, 152.9, 156.7

UV (methanol): λ_{max} 264 nm (pH 7)

<u>Experimental Example 26</u>. Synthesis of benzoic acid (2R,3S,4R,5R)-5-(6-amino-2-chloro-purin-9-yl)-3,4-dihydroxytetrahydrothiophen-4-yl methyl ester compound (26)

5 mL of 80% aqueous acetic acid solution were added to 102 mg (0.22 mmol) of benzoic acid (3aS,4R,6R,6aR)-6-(6-amino-2-chloro-purin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-

d][1,3]dioxol-4-yl methyl ester, which was then stirred at 55°C for 12 h. The reaction mixture was distilled under reduced pressure, and the pH of the concentrate was adjusted to neutral by adding saturated ammonia methanol solution. The concentrate was purified by silica gel column chromatography using the eluant (dichloromethane and methanol = 15:1) to obtain 63 mg (yield: 68%) of benzoic acid (2R,3S,4R,5R)-5-[6-amino-2-chloro-purin-9-yl]-3,4-dihydroxytetrahydrothiophen-4-yl methyl ester as white foam.

Experimental Example 27. Synthesis of (2R,3S,4R,5R)-[5-(6-amino-2-chloro-purin-9-yl)3,4-bis(tert-butyldimethylsilanyloxy)tetrahydrothiophen-2-yl]-methanol compound (27)

230 mg (0.55 mmol) of benzoic acid (2R,3S,4R,5R)-5-[6-amino-2-chloro-purin-9-yl]-3,4-dihydroxytetrahydrothiophen-4-yl methyl ester from the aforementioned Experimental Example 26, 225 mg (3.30 mmol) of imidazole, and 249 mg (1.65 mmol) of tert-butylchlorodimethylsilane were dissolved in 10 mL of N,N-dimethylformamide, which was stirred at 50°C for 24 h. Water was added to the reaction mixture, and the compound was extracted with dichloromethane. The organic layer was washed sequentially with water, saturated sodium bicarbonate solution, and saturated saline solution, then dried, filtered, and distilled under reduced pressure. Without further purification, the concentrate was dissolved in 8 mL of methanol to which 45 mg (0.83 mmol) of sodium methoxide were added. The reaction mixture was stirred at ambient temperature for 4 h, then neutralized with acetic acid and distilled under reduced pressure. The concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 2:1) to obtain 169 mg (yield: 56%) of (2R,3S,4R,5R)-[5-(6-amino-2-chloro-purin-9-yl)-3,4-bis(tert-butyldimethylsilanyloxy)tetrahydrothiophen-2-yl]-methanol as white foam.

^{1}H-NMR($CDCl_3$) δ : 0.01 (m, 12H), 0.79 (s, 9H), 0.83 (s, 9H), 3.41 (dd, 1H), 4.21 (m, 1H), 4.56 (dd, 1H), 4.73 (m, 1H), 4.86 (dd, 1H), 5.71 (d, 1H), 6.20 (br s, 1H), 7.61 (br s, 2H), 8.12 (s, 1H)

UV (methanol): λ_{max} 263 nm (pH 7)

Experimental Example 28. Synthesis of (2S,3S,4R,5R)-[5-(6-amino-2-chloro-purin-9-yl)-3,4-bis(tert-butyldimethylsilanyloxy)tetrahydrothiophene-2-carboxylic acid methyl amide compound (28)

150 mg (0.27 mmol) of (2R,3S,4R,5R)-[5-(6-amino-2-chloro-purin-9-yl)-3,4-bis(tert-butyldimethylsilanyloxy)tetrahydrothiophen-2-yl]-methanol from the aforementioned Experimental Example 27 were dissolved in 20 mL of anhydrous N,N-dimethylformamide to which 2.34 g (6.21 mmol) of pyridinium dichromate were added. The reaction mixture was

stirred at ambient temperature for 20 h. Water was added to the reaction mixture, which was then extracted with ethyl acetate several times. The organic layer was dried and filtered. The filtrate was distilled under reduced pressure to obtain the concentrate, an acid derivative. Without further purification, the concentrate was dissolved in 6 mL of acetone to which 1.0 mL (10.57 mmol) of dimethyl sulfate and 100 mg (0.72 mmol) of potassium carbonate were added, followed by stirring at ambient temperature for 2 h. The reaction mixture was distilled under reduced pressure, and the obtained concentrate was dissolved in ethyl acetate, which was washed with water and saturated saline solution. The organic layer was distilled under reduced pressure to obtain the methyl ester derivative as a foam. Without further purification, 20 mL (40 mmol) of 2N methylamine tetrahydrofuran solution were added to the aforementioned methyl ester derivative, which was stirred in a sealed container at 50°C for 24 h. After the reaction mixture was distilled under reduced pressure, the obtained concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 1:1) to obtain 80 mg (yield: 51%) of (2S,3S,4R,5R)-[5-(6-amino-2-chloro-purin-9-yl)-3,4-bis(tert-butyldimethylsilanyloxy)tetrahydrothiophene-2-carboxylic acid methyl amide as white foam.

^{1}H-NMR($CDCl_3$) δ : 0.01 (m, 12H), 0.79 (s, 9H), 0.85 (s, 9H), 2.67 (d, 3H), 3.78 (d, 1H), 4.35 (m, 1H), 4.57 (m, 1H), 5.70 (d, 1H), 7.72 (br s, 2H), 7.72 (br s, 1H), 8.10(s, 1H)

UV (methanol): λ_{max} 264 nm (pH 7)

Experimental Example 29. Synthesis of (2S,3S,4R,5R)-[5-(6-amino-2-chloro-purin-9-yl)-3,4-dihydroxytetrahydrothiophene-2-carboxylic acid methyl amide compound (29)

140 mg (0.24 mmol) of (2S,3S,4R,5R)-[5-(6-amino-2-chloro-purin-9-yl)-3,4-bis(tert-butyldimethylsilanyloxy)tetrahydrothiophen-2-yl]-methyl amide from the aforementioned Experimental Example 28 were dissolved in 10 mL of anhydrous tetrahydrofuran to which 0.67 mL (0.67 mmol, 1M tetrahydrofuran solution) of tetrabutylammonium fluoride was added. The reaction mixture was stirred at ambient temperature for 1 h. The reaction mixture was distilled under reduced pressure and the concentrate was purified by silica gel column chromatography using the eluant (dichloromethane:methanol = 4:1) to obtain 58 mg (yield: 69%) of (2S,3S,4R,5R)-5-(6-amino-2-chloro-purin-9-yl)-3,4-dihydroxytetrahydrothiophene-2-carboxylic acid methyl amide as white solids (see Table 3).

Melting point: 233-235°C

$[\alpha]^{26}_D$: -20.2 (c 0.1 methanol)

^{1}H-NMR(DMSO-d_6) δ : 2.70(d, 3H), 3.82 (d, 1H), 4.36 (dd, 1H), 4.53 (m, 1H), 5.60 (d, 1H), 5.78 (d, 1H), 5.82 (d, 1H), 7.87 (br s, 2H), 8.33 (br d, 1H), 8.55 (s, 1H)

^{13}C-NMR(DMSO-d_6): δ 51.8, 57.5, 62.5, 75.4, 78.1, 118.4, 140.1, 150.5, 153.0, 156.7, 170.7

UV (methanol): λ_{max} 264 nm (pH 7)

Experimental Example 30. Synthesis of benzoic acid (2R,3S,4R,5R)-5-(2-chloro-6-methylaminopurin-9-yl)-3,4-dihydroxytetrahydrothiophen-2-yl methyl ester compound (30)

5 mL of 80% aqueous acetic acid solution were added to 105 mg (0.22 mmol) of benzoic acid (3aS,4R,6R,6aR)-6-(2-chloro-6-methylaminopurin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester, which was then stirred at 55°C for 12 h. The reaction mixture was distilled under reduced pressure, then neutralized by adding saturated ammonia methanol solution. The concentrate was purified by silica gel column chromatography using the eluant (dichloromethane and methanol = 15:1) to obtain 93 mg (yield: 68%) of benzoic acid (2R,3S,4R,5R)-5-[2-chloro-6-methylaminopurin-9-yl]-3,4-dihydroxytetrahydrothiophen-2-yl methyl ester as white foam.

Experimental Example 31. Synthesis of (2R,3S,4R,5R)-[3,4-bis(tert-butyldimethylsilanyloxy)-5-(2-chloro-6-methylaminopurin-9-yl)-tetrahydrothiophen-2-yl]-methanol compound (31)

160 mg (0.38 mmol) of benzoic acid (2R,3S, 4R,5R)-5-[2-chloro-6-methylaminopurin-9-yl]-3,4-dihydroxytetrahydrothiophen-2-yl methyl ester from the aforementioned Experimental Example 30, 155 mg (2.28 mmol) of imidazole, and 172 mg (1.14 mmol) of tert-butylchlorodimethylsilane were dissolved in 10 mL of N,N-dimethylformamide, which was stirred at 50°C for 24 h. Water was added to the reaction mixture, and the compound was extracted with dichloromethane. The organic layer was washed sequentially with water, saturated sodium bicarbonate solution, and saturated saline solution, then dried, filtered, and distilled under reduced pressure. Without further purification, the concentrate was dissolved in 8 mL of methanol to which 45 mg (0.83 mmol) of sodium methoxide were added. The reaction mixture was stirred at ambient temperature for 4 h, then neutralized with acetic acid and distilled under

reduced pressure. The concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 2:1) to obtain 112 mg (yield: 53%) of (2R,3S,4R,5R)-[3,4-bis(tert-butyldimethylsilanyloxy)-5-(2-chloro-6-methylaminopurin-9-yl)-tetrahydrothiophen-2-yl]-methanol as white foam.

^{1}H-NMR($CDCl_3$) δ : 0.01 (m, 12H), 0.81 (s, 9H), 0.86 (s, 9H), 3.13 (br s, 3H), 3.72 (dd, 1H), 4.24 (m, 1H), 4.48 (dd, 1H), 4.75 (m, 1H), 4.87 (dd, 1H), 5.67 (d, 1H), 5.88 (br s, 1H), 8.00 (s, 1H), 8.03 (br s, 1H)

UV (methanol): λ_{max} 269 nm (pH 7)

<u>Experimental Example 32</u>. Synthesis of (2S,3S,4R,5R)-3,4-bis(tert-butyldimethylsilanyloxy)-5-(2-chloro-6-methylaminopurin-9-yl)-tetrahydrothiophene-2-carboxylic acid methyl amide compound (32)

105 mg (0.19 mmol) of (2R,3S,4R,5R)-[3,4-bis(tert-butyldimethylsilanyloxy)-5-(2-chloro-6-methylaminopurin-9-yl)-tetrahydrothiophen-2-yl]-methanol from the aforementioned Experimental Example 31 were dissolved in 15 mL of anhydrous N,N-dimethylformamide to which 1.64 g (4.36 mmol) of pyridinium dichromate were added. The reaction mixture was stirred at ambient temperature for 20 h. Water was added to the reaction mixture, which was extracted with ethyl acetate several times. The organic layer was dried and filtered. The filtrate was distilled under reduced pressure to obtain the concentrate, an acid derivative. Without further purification, the concentrate was dissolved in 4 mL of acetone to which 1.0 mL (10.57 mmol) of dimethyl sulfate and 80 mg (10.58 mmol) of potassium carbonate were added, followed by stirring at ambient temperature for 2 h. The reaction mixture was distilled under reduced pressure, and the obtained concentrate was dissolved in ethyl acetate, then washed with water and saturated saline solution. The organic layer was distilled under reduced pressure to obtain the methyl ester derivative as foam. Without further purification, 15 mL (30 mmol) of 2N methylamine tetrahydrofuran solution were added to the aforementioned methyl ester derivative, then it was stirred in a sealed container at 50°C for 24 h. After the reaction mixture was distilled under reduced pressure, the obtained concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 1:1) to obtain 48 mg (yield: 44 %) of (2S,3S,4R,5R)-3,4-bis(tert-butyldimethylsilanyloxy)-5-(2-chloro-6-methylaminopurin-9-yl)-tetrahydrothiophene-2-carboxylic acid methyl amide as white foam.

^{1}H-NMR($CDCl_3$) δ : 0.01 (m, 12H), 0.82 (s, 9H), 0.85 (s, 9H), 3.21 (br s, 3H), 3.21 (br s, 3H), 3.75 (d, 1H), 4.28 (m, 1H), 4.65 (m, 1H), 5.77 (d, 1H), 7.12 (br s, 1H), 8.03 (br s, 1H), 8.44 (s, 1H)

UV (methanol): λ_{max} 270 nm (pH 7)

Experimental Example 33. Synthesis of (2S,3S,4R,5R)- 5-(2-chloro-6-methylaminopurin-9-yl)-3,4-dihydroxytetrahydrothiophene-2-carboxylic acid methyl amide compound (33)

48 mg (0.08 mmol) of (2S,3S,4R,5R)-3,4-bis(tert-butyldimethylsilanyloxy)-5-(2-chloro-6-methylaminopurin-9-yl)-tetrahydrothiophene-2-carboxylic acid methyl amide were dissolved in anhydrous tetrahydrofuran to which 0.22 mL (0.22 mmol, 1M tetrahydrofuran solution) of tetrabutylammonium fluoride was added. The reaction mixture was stirred at ambient temperature for 1 h. The reaction mixture was distilled under reduced pressure and the concentrate was purified by silica gel column chromatography using the eluant (dichloromethane:methanol = 6:1) to obtain 19 mg (yield: 65%) of (2S,3S,4R,5R)-5-(2-chloro-6-methylaminopurin-9-yl)-3,4-dihydroxytetrahydrothiophene-2-carboxylic acid methyl amide as white solids (see Table 3).

Melting point: 130-141°C

$[\alpha]^{19}_D$: -22.0 (c 0.13 methanol)

^{1}H-NMR(DMSO-d_6) δ : 2.69 (d, 3H), 2.92 (d, 3H), 3.81 (d, 1H), 4.36 (dd, 1H), 4.52 (dd, 1H), 5.61 (d, 1H), 5.78 (d, 1H), 5.82 (d, 1H), 7.23 (br s, 1H), 8.33 (br d, 2H), 8.54 (s, 1H)

^{13}C-NMR(DMSO-d_6): δ 51.8, 55.5, 62.6, 69.6, 75.4, 78.2, 118.5, 139.9, 149.5, 153.3, 155.5, 170.7

UV (methanol): λ_{max} 270 nm (pH 7)

Experimental Example 34. Synthesis of (2R,3S, 4R,5R)-{3,4-bis(tert-butyldimethylsilanyloxy)-5-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-tetrahydrothiophen-2-yl} methanol compound (34)

6 mL of 80% aqueous acetic acid solution were added to 122 mg (0.18 mmol) of benzoic acid (3aS,4R,6R,6aR)-6-(2-chloro-6-(3-iodobenzylamino)purin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl methyl ester, which was then stirred at 55°C for 12 h. The reaction mixture was distilled under reduced pressure, then adjusted to neutral by adding saturated ammonia methanol solution. The concentrate was purified by silica gel column chromatography using the eluant (dichloromethane and methanol = 15:1) to obtain benzoic acid (2R,3S,4R,5R)-5-[2-chloro-6-(iodobenzylamino)purin-9-yl]-3,4-dihydroxytetrahydrothiophen-2-yl methyl ester as white foam. 450 mg (0.71 mmol) of benzoic acid (2R,3S, 4R,5R)-5-[2-chloro-6-(iodobenzyyamino)purin-9-yl]-3,4-dihydroxytetrahydrothiophen-2-yl methyl ester obtained above, 286 mg (4.20 mmol) of imidazole, and 316 mg (2.10 mmol) of tert-butylchlorodimethylsilane were dissolved in 20 mL of anhydrous N,N-dimethylformamide, which was then stirred at 50°C for 24 h. Water was added to the reaction mixture, which was extracted with dichloromethane. The organic layer was washed sequentially with water, saturated sodium bicarbonate solution, and then saturated saline solution. The organic layer was dried and then distilled under reduced pressure. Without further purification, the concentrate was dissolved in 15 mL of methanol to which 90 mg (1.74 mmol) of sodium methoxide were added. The reaction mixture was stirred at ambient temperature for 4 h and the solution was neutralized with acetic acid, then distilled under reduced pressure. The concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 2:1) to obtain 290 mg (yield: 54%) of (2R,3S,4R,5R)-{3,4-bis(tert-butyldimethylsilanyloxy)-5-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-tetrahydrothiophen-2-yl} methanol as white solids.

^{1}H-NMR($CDCl_3$) δ : 0.01 (m, 12H), 0.80 (s, 9H), 0.85 (s, 9H), 3.29 (dd, 1H), 4.24 (m, 1H), 4.47 (dd, 1H), 4.73 (m, 3H), 4.86 (dd, 1H), 5.67 (d, 1H), 6.15 (br s, 1H), 7.02 (t, 1H), 7.27 (d, 1H), 7.43 (d, 1H), 7.59 (br s, 1H), 7.67 (s, 1H), 8.03 (s, 1H)

UV (methanol): λ_{max} 272 nm (pH 7)

Experimental Example 35. Synthesis of (2S,3S,4R,5R)-3,4-bis(tert-butyldimethylsilanyloxy)-5-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-tetrahydrothiophene-2-carboxylic acid methyl amide compound (35)

228 mg (0.30 mmol) of (2R,3S,4R,5R)-[3,4-bis(tert-butyldimethylsilanyloxy)-5-(2-chloro-6-(3-iodobenzylamino)purin-9-yl)-tetrahydrothiophen-2-yl]-methanol were dissolved in

20 mL of anhydrous N,N-dimethylformamide to which 2.6 g (7.0 mmol) of pyridinium dichromate were added. The reaction mixture was stirred at ambient temperature for 20 h. Water was added to the reaction mixture, which was then extracted with ethyl acetate several times. The organic layer was dried and filtered. The filtrate was distilled under reduced pressure to obtain the concentrate, an acid derivative. Without further purification, the concentrate was dissolved in 6 mL of acetone to which 1.0 mL (10.57 mmol) of dimethyl sulfate and 100 mg (0.72 mmol) of potassium carbonate were added, followed by stirring at ambient temperature for 2 h. The reaction mixture was distilled under reduced pressure, and the obtained concentrate was dissolved in ethyl acetate, then washed with water and saturated saline solution. The organic layer was distilled under reduced pressure to obtain the methyl ester derivative as foam. Without further purification, 20 mL (40 mmol) of 2N methylamine tetrahydrofuran solution were added to the aforementioned methyl ester derivative, then it was stirred in a sealed container at 50°C for 24 h. After the reaction mixture was distilled under reduced pressure, the obtained concentrate was purified by silica gel column chromatography using the eluant (hexane:ethyl acetate = 1:2) to obtain 99 mg (yield: 42%) of (2S,3S,4R,5R)-3,4-bis(tert-butyldimethylsilanyloxy)-5-(2-chloro-6-(3-iodobenzylaminopurin-9-yl)-tetrahydrothiophene-2-carboxylic acid methyl amide as white foam.

^{1}H-NMR(CDCl$_3$) δ : 0.01 (m, 12H), 0.70 (s, 9H), 0.84 (s, 9H), 2.70 (d, 3H), 3.81 (d, 1H), 4.39 (m, 1H), 4.55 (m, 1H), 4.61 (d, 2H), 5.83 (d, 1H), 7.15 (t, 1H), 7.36 (d, 1H), 7.61 (d, 1H), 7.76 (s, 1H), 8.33 (br s, 1H), 8.61 (d, 1H), 9.00 (br s, 1H)

UV (methanol): λ_{max} 272 nm (pH 7)

Experimental Example 36: Synthesis of (2S,3S,4R,5R)-[5-(2-chloro-6-(3-iodobenzylamino)purin-9-yl)-3,4-dihydroxytetrahydrothiophene-2-carboxylic acid methyl amide compound (36)

75 mg (0.09 mmol) of (2S,3S,4R,5R)-3,4-bis(tert-butyldimethylsilanyloxy)-5-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-tetrahydrothiophene-2-carboxylic acid methyl amide from the aforementioned Experimental Example 35 were dissolved in 5 mL of anhydrous tetrahydrofuran to which 0.25 mL (0.25 mmol, 1 mol tetrahydrofuran solution) of tetrabutylammonium fluoride was added. The reaction mixture was stirred at ambient temperature for 1 h. The reaction mixture was distilled under reduced pressure and the concentrate was purified by silica gel column

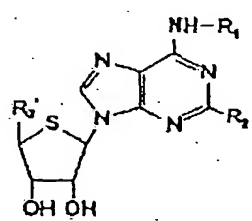
chromatography using the eluant (dichloromethane:methanol = 7:1) to obtain 33 mg (yield: 63%) of (2S,3S,4R,5R)-5-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-3,4-dihydroxytetrahydrothiophene-2-carboxylic acid methyl amide as white solids (see Table 3).

Melting point: 140-141°C

$[\alpha]^{5}_{D}$: -19.5 (c 0.32 methanol)

^{1}H-NMR(DMSO-d_6) δ : 2.69 (d, 3H), 3.82 (d, 1H), 4.37 (dd, 1H), 4.55 (m, 1H), 4.60 (d, 2H), 5.59 (d, 1H), 5.78 (d, 1H), 5.83 (d, 1H), 7.13 (t, 1H), 7.35 (d, 1H), 7.60 (d, 1H), 7.75 (s, 1H), 8.32 (br d, 1H), 8.60 (s, 1H), 8.99 (br t, 1H)

^{13}C-NMR(DMSO-d_6): δ 42.5, 51.8, 59.7, 62.6, 75.4, 78.2, 118.4, 126.8, 130.5, 135.5, 136.0, 140.3, 141.8, 149.9, 153.0, 154.7, 170.3, 170.7 UV (methanol): λ 272 nm (pH7)



(III)

Table 3

① 화합물군	② 화합물	R_1	R_2	R_3	R_3'	⑧ 스펙트럼 데이터
III	20	3-요오도벤질 ③	염소 ⑥	수소 ⑤	히드록시메틸 ⑦	^{1}H-NMR(DMSO-d_6) δ : 3.29 (m, 1H), 3.62 (m, 1H), 3.78 (m, 1H), 4.20 (d, 1H), 4.61 (m, 3H), 5.17 (t, 1H), 5.34 (d, 1H), 5.58 (d, 1H), 5.77 (d, 1H), 7.13 (t, 1H), 7.34 (d, 1H), 7.59 (d, 1H), 7.74 (s, 1H), 8.53 (s, 1H), 8.92 (br t, 1H)
	23	메틸 ④	염소 ⑥	수소 ⑤	히드록시메틸 ⑦	^{1}H-NMR(DMSO-d_6) δ : 2.92 (d, 3H), 3.30 (br s, 1H), 3.62 (m, 1H), 3.79 (m, 1H), 4.20 (dd, 1H), 4.61 (m, 1H), 5.16 (t, 1H), 5.34 (d, 1H), 5.58 (d, 1H), 5.77 (d, 1H), 8.26 (br s, 1H), 8.47 (s, 1H)
	25	수소 ⑤	염소 ⑥	수소 ⑤	히드록시메틸 ⑦	^{1}H-NMR(DMSO-d_6) δ : 3.30 (br s, 1H), 3.62 (m, 1H), 3.79 (m, 1H), 4.20 (dd, 1H), 4.61 (dd, 1H), 5.16 (t, 1H), 5.34 (d, 1H), 5.58 (d, 1H), 5.77 (d, 1H), 7.81 (br s, 2H), 8.48 (s, 1H)

Key:

1 Compound group
2 Compound
3 3-iodobenzyl
4 Methyl
5 Hydrogen
6 Chlorine
7 Hydroxymethyl
8 Spectral data

① 화합물군	② 화합물	R_1	R_2	R_3	R_3'	⑧ 스펙트럼 데이터
Ⅲ	29	수소 ③	염소 ⑥	수소 ③	메틸아미노카르보닐 ⑦	^{1}H-NMR(DMSO-d_6) δ : 2.70(d, 3H), 3.82 (d, 1H), 4.36 (dd, 1H), 4.53 (m, 1H), 5.60 (d, 1H), 5.78 (d, 1H), 5.82 (d, 1H), 7.87 (br s, 2H), 8.33 (br d, 1H), 8.55 (s, 1H)
	33	메틸 ④	염소 ⑥	수소 ③	메틸아미노카르보닐 ⑦	^{1}H-NMR(DMSO-d_6) δ : 2.69 (d, 3H), 2.92 (d, 3H), 3.81 (d, 1H), 4.36 (dd, 1H), 4.52 (dd, 1H), 5.61 (d, 1H), 5.78 (d, 1H), 5.82 (d, 1H), 7.23 (br s, 1H), 8.33 (br d, 2H), 8.54 (s, 1H)
	36	3-요오도벤질 ⑤	염소 ⑥	수소 ③	메틸아미노카르보닐 ⑦	^{1}H-NMR(DMSO-d_6) δ : 2.69 (d, 3H), 3.82 (d, 1H), 4.37 (dd, 1H), 4.55 (m, 1H), 4.60 (d, 2H), 5.59 (d, 1H), 5.78 (d, 1H), 5.83 (d, 1H), 7.13 (t, 1H), 7.35 (d, 1H), 7.60 (d, 1H), 7.75 (s, 1H), 8.32 (br d, 1H), 8.60 (s, 1H), 8.99 (br t, 1H)

Key:
1 Compound group
2 Compound
3 Hydrogen
4 Methyl
5 3-iodobenzyl
6 Chlorine
7 Methylaminocarbonyl
8 Spectral data

Test Example 1. Binding affinity test of 4-thioadenosine derivative compound

Cell culture and receptor binding

Chinese hamster ovary (CHO, ATCC; US Cell Strain Bank No. CCL-61) cells that express the A_3 receptor were cultured at 37°C in the presence of 5% carbon dioxide in F-12 culture medium (Gibco Company, USA) containing 10% fetal bovine serum (FBS) and penicillin/streptomycin (100 U/mL and 100 μg/mL), and used.

The binding affinity for the CHO cell membrane [^{125}I]-4-amino-3-iodobenzyl]adenosine-5'-N-methyluronamide ([^{125}I]AB-MECA) was measured in a test tube holding 50/10/1 buffer solution which contains 50 μL of [^{125}I]AB-MECA, 100 μL of membrane suspended particle, and 50 μL of inhibitor. The inhibitor is first dissolved in dimethyl sulfoxide and diluted with buffer solution. The final concentration of DMSO should not exceed 1%. After culturing at 37°C for 1 h, it was quickly filtered using a cell collector (TOMTEC Company, USA) and a GF/B filter (Whatman Company, USA). The test tube was rinsed three times with 3 mL of buffer solution,

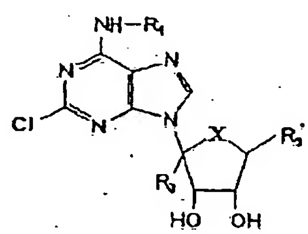
and the radioactivity was measured using a γ-counter. Nonspecific binding was determined in the presence of 40 μM of R-PIA and the equilibrium constant, K_i, was determined using the Cheng-Prusoff equation under the assumption that the k_d value of [^{125}I]AB-MECA is 1.48 nM.

The binding of [^{3}H]PIA((R)-N^6-(phenylisopropyl)adenosine) to A_1 and the binding of [^{3}H]CGS21680(2-[[[4-(2-carboxyethyl-phenyl)ethylamino]-5'-N-ethylcarbamoyl)adenosine)* to A_{2a} were measured as described below. Adenosine deaminase was added while the membrane was cultured at 30°C for 30 min with the radioactive ligand. The IC_{50} values of each compound were determined for at least 6 different concentrations. This value was used to determine the K_i value using the Plat program, wherein the Cheng-Prusoff equation was used under the assumption that the K_d values of [^{3}H]PIA and [^{3}H]CGS 21680 are 1.0 and 14 nM, respectively.

Among the known adenosine derivatives, CI-IB-MECA used as a control group herein is known to exhibit outstanding agonist activity against A_3 among A_1, A_2, and A_3, but very low affinity toward A_1 and A_2 (Hea O. Kim, et al.; J. Med. Chem., 37(21), pp. 364-3621, 1994).

The test results showed that, among new thionucleoside compounds of the present invention, K_i toward adenosine A_3 receptor of the compounds having the general formula (III) was in the range of 0.28-4.9 nM, indicating strong agonist activity compared to the existing human A_3 receptors. K_i of the compound having the general formula (II) was found to be 4.3-8.0 nM, which means excellent antagonist activity. The compound having general formula (II) showed much lower affinity toward A_1 and A_2 than the existing compounds. Especially, compound 33 exhibited the most strong and selective A_3 agonist activity among the known compounds.

As a result, it was confirmed that these compounds can be used for the treatment of cancer and inflammatory diseases through the adenosine A_3 receptor activity because these compounds showed excellent selectivity toward adenosine A_3 receptors.



(V)

Table 4

* [Editor's note: The incorrect use of parentheses or brackets follows the original document.]

① 화합물	화합물군 ②	R_1	R_3	R_3'	③ Ki (nM) 또는 %				
					hA_1	rA_1	hA_{2A}	rA_{2A}	hA_3
Cl-IB-MECA		④ 3-요오도벤질	수소 ⑤	CONHMe	1240 ± 320	820± 570	5360 ± 2470	470± 365	1.0± 0.2
20	III	④ 3-요오도벤질	수소 ⑤	CH_2OH		127± 35		285± 108	3.2± 0.9
25	III	⑤ 수소	수소	CH_2OH		238± 107		705± 184	4.9± 1.3
23	III	⑥ 메틸	수소 ⑤	CH_2OH		780± 280		<10%(10 μM)	0.8± 0.1
12	II	④ 3-요오도벤질	CONHMe	수소 ⑤	110± 12	188± 40	50%(10 μM)	<10%(10 μM)	4.3± 1.2
33	III	⑥ 메틸	수소 ⑤	CONHMe	1330± 242	198± 14	20%(10 μM)	6340± 90	0.28 ± 0.09
36	III	④ 3-요오도벤질	수소 ⑤	CONHMe	193± 46	140± 43	223± 36	345± 110	0.38 ± 0.07
29	III	⑤ 수소	수소 ⑤	CONHMe	89.2 ± 11.7	294± 115	158± 29	<10%(10 μM)	0.40 ± 0.06

Key:
1 Compound
2 Compound group
3 K_i (nM) or %
4 3-iodobenzyl
5 Hydrogen
6 Methyl

Test Example 2. Toxicity test

Animal studies were carried out to test the toxicity of the compounds prepared in the present invention.

25 ± 5 g ICR family mice (Joongang Study Animal) and 235 ± 10 g specific pathogen free (SPF) Sprague Dawley rats (Joongang Study Animal) were divided into 3 groups. After 20 mg/kg, 10 mg/kg, and 1 mg/kg dose of compound 33 of the present invention were peritoneally administered, the toxicity was observed for 24 h.

In the test results, no deaths were observed in any of three groups, and there were no other observable symptoms related to weight increase or feed intake, etc., that were different from the control group. Therefore, it was confirmed that the thionucleoside derivative compounds are safe drugs.

The thionucleoside derivative compounds of the present invention may be administered in the following formulations. The following formulation examples are listed only to illustrate the present invention. The content of the present invention is not limited to these examples.

Formulation Example 1. Powder formulation

Dry powder compound 33	500 mg
Cornstarch	100 mg
Lactose	100 mg
Talc	100 mg

The ingredients listed above were mixed and loaded into airtight sacks, and powder was prepared.

Formulation Example 2. Tablet formulation

Dry powder of compound 33	100 mg
Cornstarch	100 mg
Lactose	100 mg
Magnesium stearate	2 mg

After the ingredients listed above were mixed together, tablets were made using the conventional tablet manufacturing method.

Formulation Example 3. Capsule formulation

Dry powder of compound 33	50 mg
Lactose	50 mg
Magnesium stearate	1 mg

The ingredients listed above were mixed, then loaded into gelatin capsules made by conventional capsule manufacturing method.

Formulation Example 4. Injection formulation

Dry powder of compound 33	10 mg
Sterilized distilled water for injection	Appropriate amount
pH adjusting agent	Appropriate amount

According to the manufacturing method for conventional injectable solutions, the active ingredient was dissolved in distilled water for injection and the pH of the solution was adjusted to approximately 7.5. The entire solution was loaded into a 2-mL capacity ampule using distilled water for injection, which was then sterilized to prepare the injectable solution.

Formulation Example 5. Liquid formulation

Dry powder of compound 33	1g
Isomerized [sic] saccharide	10 g
Saccharide	10 g

Lemon flavor	Appropriate amount
Purified water	Appropriate amount

According to conventional manufacturing methods for liquid drugs, each ingredient was added to purified water and dissolved. After an appropriate amount of the lemon flavor was added and the entire solution was brought to 100 mL by adding the purified water, it was then loaded in a brown bottle and sterilized to prepare a liquid drug.

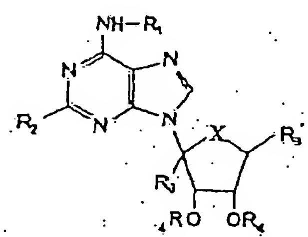
The aforementioned composition ratio is presented as a preferred example wherein ingredients suitable for beverages are mixed. However, the composition ratio may be altered according to the preferred regional and ethnic taste depending on consumer class, consumer country, application, etc.

Effect of the invention

New thionucleoside derivative compounds of the present invention and pharmaceutical compositions containing these compounds have selectivity for adenosine A_3 receptors, and therefore they can be used as effective drugs for preventing and treating various cancers and inflammatory intestinal diseases and inflammatory diseases.

Claims

1. Compounds having the following general formula (I) or their pharmaceutically acceptable salts or isomers:



(I)

wherein

X is sulfur or oxygen;

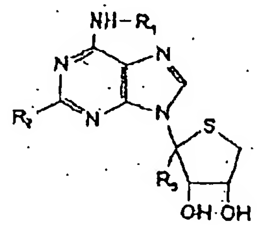
R_1 is a hydrogen, alkyl group with 1-5 carbons, benzyl group, halobenzyl group, or the phenylalkyl group;

R_2 is a hydrogen, halogen group, alkoxy group, alkenyl group, alkynyl group, alkylthio group or thio group;

R_3 and R_3' are hydroxyalkyl groups containing 1-5 carbons, alkoxycarbonyl groups, or alkylaminocarbonyl groups containing 1-5 carbons, wherein R_3 and R_3' are not the same;

R_4 is hydrogen or alkyl group containing 1-5 carbons.

2. Compounds having the following general formula (II) or isomers as claimed in Claim 1, which has hydrogen for R_3' and R_4 and sulfur for X,



(II)

wherein

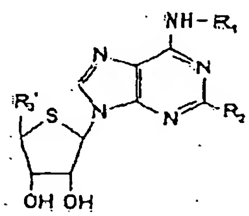
R_1 is a hydrogen, alkyl group having 1-5 carbons, benzyl group, halobenzyl group or phenylalkyl group,

R_2 is a hydrogen, halogen group, alkoxy group, alkenyl group, alkynyl group, alkylthio group or thio group,

R_3 is a hydroxyalkyl group having 1-5 carbons, alkoxycarbonyl group, or alkylaminocarbonyl group having 1-5 carbons.

3. (2R,3S,4R)-2-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-3,4-dihydroxytetrahydrothiophene-2-carboxylic acid methyl amide compound as claimed in Claim 2.

4. Compounds having the following general formula (III) or isomers as claimed in Claim 1, which has hydrogen for R_3 and R_4 and sulfur for X,



(III)

wherein

R_1 is a hydrogen, alkyl group having 1-5 carbons, benzyl group, halobenzyl group, or phenylalkyl group,

R_2 is a hydrogen, halogen group, alkoxy group, alkenyl group, alkynyl group, alkylthio group, or thio group,

R_3' is a hydroxyalkyl group having 1-5 carbons, alkoxycarbonyl group, or alkylaminocarbonyl group having 1-5 carbons.

5. (2R,3R,4S,5R)-2-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-5-hydroxymethyltetrahydrothiophene-3,4-diol,

(2R,3R,4S,5R)-2-(2-chloro-6-methylaminopurin-9-yl]-5-hydroxymethyltetrahydrothiophene-3,4-diol,

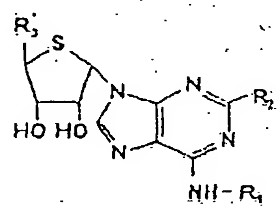
(2R,3R,4S,5R)-2-(2-chloro-6-methylaminopurin-9-yl]-5-hydroxymethyltetrahydrothiophene-3,4-diol,

(2S,3S,4R,5R)-5-(6-amino-2-chloro-purin-9-yl]-3,4-dihydroxytetrahydrothiophene-2-carboxylic acid methyl amide,

(2S,3S,4R,5R)-5-(2-chloro-6-methylaminopurin-9-yl]-3,4-dihydroxytetrahydrothiophene-2-carboxylic acid methyl amide,

(2S,3S,4R,5R)-5-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-3,4-dihydroxytetrahydrothiophene-2-carboxylic acid methyl amide compounds of Claim 4.

6. Compounds according to Claim 1 having the general formula (IV) or isomers having hydrogen for R_3 and R_4 and sulfur for X,



(IV)

wherein

R_1 is a hydrogen, alkyl group having 1-5 carbons, benzyl group, halobenzyl group, or phenylalkyl group,

R_2 is a hydrogen, halogen group, alkoxy group, alkenyl group, alkynyl group, alkylthio group, or thio group,

R_3' is a hydroxyalkyl group having 1-5 carbons, alkoxycarbonyl group, or alkylaminocarbonyl group having 1-5 carbons.

7. (2S,3R,4S,5R)-2-[2-chloro-6-(3-iodobenzylamino)purin-9-yl]-5-hydroxymethyltetrahydrothiophene-3,4-diol compound of Claim 6.

8. Pharmaceutical compositions with selectivity for adenosine A_3 receptors, which contain the compound having one of the general formulas claimed in Claims 1-7 as an active ingredient and pharmaceutically acceptable carriers.

9. Pharmaceutical compositions of Claim 8, which are effective for preventing and treating cancers or inflammatory diseases by selectivity for adenosine A_3 receptors.

(CLAIM 10)

This invention contains the preparations composed medically effective ingredients out of CLAIM 1-7 on the treatment for cancer diseases and pharmacologically acceptable carriers.

(CLAIM 11)

Cancer diseases in CLAIM 10 contains lung cancer, skin cancer.

(CLAIM 12)

This invention contains the preparations composed medically effective ingredients out of CLAIM 1-7 on the treatment for inflammatory diseases and pharmacologically acceptable carriers.

N^6-Substituted D-4'-Thioadenosine-5'-methyluronamides: Ultrapotent and Selective Agonists at the Human A_3 Adenosine Receptor

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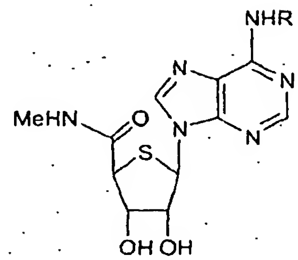
N^6-Substituted D-4′-Thioadenosine-5′-methyluronamides: Ultrapotent and Selective Agonists at the Human A_3 Adenosine Receptor

Lak Shin Jeong* Dong Zhe Jin, Hyung Ryong Moon, Hea Ok Kim, Dae Hong Shin, Moon Woo Chun, Neli Melman, Zhan-Guo Gao, and Kenneth A. Jacobson



R = H, Me, 3-iodobenzyl

Abstract: On the basis of bioisosteric rationale, 4'-thio analogues of Cl-IB-MECA (2-chloro-N^6-(3-iodobenzyl)adenosine-5'-methyluronamide), one of the most potent and selective agonists (K_i = 1.0 ± 0.2 nM) at the human A_3 adenosine receptor, was efficiently synthesized starting from D-gulono γ-lactone via 4-thioribosyl acetate **14** as the key intermediate. All synthesized 4'-thionucleosides exhibited higher binding affinity to the human A_3 adenosine receptor than Cl-IB-MECA, among which the 2-chloro-N^6-methyladenosine-5'-methyluronamide showed the most potent binding affinity (K_i = 0.28 ± 0.09 nM). It was selective for A_3 vs rat A_1 and rat A_{2A} receptors by 700- and 23,000-fold, respectively and for A_3 vs human A_1 and human A_{2A} receptors by 4,800- and 36,000-fold, respectively.

Introduction. Adenosine is known to be involved in regulation of many physiological functions through specific adenosine receptors, which are expressed on the surface of nearly all cells.[1] Four subtypes of adenosine receptors have now been cloned, characterized pharmacologically and classified as A_1, A_{2A}, A_{2B}, and A_3. Since these receptors have pharmacological effects relating to cardiovascular diseases,[2] central nervous system (CNS) diseases,[3] inflammation,[4,5] renal failure,[6]

Parkinson's and Huntington's diseases,[7] they have been promising targets for the development of new therapeutic agents. Among these, the A_3 adenosine receptor was the most recently identified and cloned from a rat brain cDNA library, and later from sheep and human brain.[8-10] There is low sequence homology among A_3 adenosine receptors of different species, with rat and human being only 72% identical and human and sheep being 85% identical, which is consistent with large interspecies differences in binding affinities for antagonists and agonists.[11] The A_3 adenosine receptors are G protein-coupled receptors and their activation inhibits adenylate cyclase[1] and stimulates phospholipase C,[12] leading to protein kinase C activation and histamine release[13] from rat mast cells. A_3 adenosine receptor agonists are under consideration in the treatment of cardiac and cerebral ischemia and cancer,[2,3,12] while A_3 adenosine receptor antagonists have been suggested to be useful as a potential treatment for glaucoma and possibly inflammation.[13]

A number of ligands have been synthesized and tested for binding affinity at the rat, sheep, and human A_3 versus A_1 and A_{2A} receptors. Among these ligands, IB-MECA (**1**) was found to be a highly potent rat A_3

agonist (K_i = 1.1 nM), which is 50-fold selective for rat brain A_3 versus either A_1 or A_2 receptors.[14] Introduction of chlorine at the 2-position of IB-MECA, resulting in the formation of Cl-IB-MECA (**2**) dramatically increased binding affinity and selectivity.[14] Compound **2** has been reported to display a K_i value of 0.33 nM and showed 2500- and 1400-fold rat A_3 receptor selectivity versus A_1 and A_{2A} receptors, respectively. Compound **2** is now being used extensively as a pharmacological tool for studying A_3 receptors.[15] Therefore, based on the high binding affinity and selectivity of **2** on A_3 adenosine receptors, we were interested in designing and synthesizing the 4'-thio analogue **3** of Cl-IB-MECA (**2**) and its related analogues **4** and **5**, since a sulfur atom may serve as a bioisostere of an oxygen atom. A new ligand, thio-Cl-IB-MECA (**3**) and its derivatives **4** and **5** were efficiently synthesized from D-gulonic γ-lactone using novel and highly efficient synthetic procedure to prepare the 4-thioribose derivative. The synthesized 4'-thionucleoside derivatives exhibited dramatic increases in affinity and selectivity for human A_3 receptors versus rat and human A_1 and A_{2A} receptors. In this communication, we report the N^6-substituted 4'-thionucleoside-5'-methyluronamides, **3**, **4**, and **5** as ultrapotent and

selective agonists at the human A_3 receptor. Such agonists may be useful as pharmacological tools and also are of interest for development as therapeutic agents.

Results and discussion

Chemistry. The synthetic strategy to the desired nucleosides was to synthesize the glycosyl donor and then to condense with nucleobases.

Scheme 1

Synthesis of the glycosyl donor **14**, starting from D-gulonic γ-lactone (**6**) is shown in Scheme 1. Starting material **6** was converted to the diacetonide **7** under the standard reaction conditions. Reduction of **7** with LAH followed by mesylation of the resulting diol afforded the dimesylate **8**. Cyclization of **8** with anhydrous sodium sulfide smoothly proceeded upon heating in DMF to yield the thiosugar **9** in 94% yield. Selective hydrolysis of the 5,6-acetonide in the presence of 2,3-acetonide was achieved using 30% aqueous acetic acid to give diol **10**. Oxidative cleavage of **10** with lead tetraacetate followed by reduction of the resulting aldehyde with $NaBH_4$ afforded **11**, which was treated with benzoyl chloride to give the benzoate **12**. Oxidation of **12** with *m*CPBA followed by heating the sulfoxide **13** with

acetic anhydride produced the key intermediate **14**.

The glycosyl donor **14** was utilized for the synthesis of 2-chloro-N^6-substituted-4'-thiopurine nucleosides **3**, **4**, and **5** as shown in Scheme 2.

Scheme 2

The glycosyl donor **14** was condensed with silylated 2,6-dichloropurine in the presence of TMSOTf to give the β-anomer **15** and its α-anomer in 4:1 ratio. Anomeric configurations were easily assigned by ^{1}H NOE experiment. The β-anomer **15** was converted to **17**, **18**, and **19** by treating with 3-iodobenzylamine, methylamine, and ammonia, respectively. For the synthesis of the uronamide derivatives, compounds **17**, **18**, and **19** were treated with 80% aqueous acetic acid to give the diol derivatives, which were protected as disilyl ethers and then debenzoylated to afford the 4'-hydroxymethyl analogues **20**, **21**, and **22**, respectively. Oxidation of **20**, **21**, and **22** with PDC in DMF followed by esterification with potassium carbonate and dimethyl sulfate in acetone, conversion of the 5'-esters to 5'-uronamides by treating with 40% methylamine, and the final desilylation yielded the final nucleosides **3**, **4**, and **5**, respectively.

Biological activity. The synthesized analogues were tested in radioligand

binding assays[16] using rat cortical A_1 receptors or striatal A_{2A} receptors or in CHO cells stably expressing the recombinant receptors (human A_1, A_{2A}, or A_3). Radioligands for A_1, A_{2A}, and A_3 receptors were the selective agonists [^{3}H]-N^6-(*R*)-phenylisopropyladenosine (PIA) (A_1), [^{3}H]CGS21680 (A_{2A}), and [^{125}I]-I-AB-MECA [N^6-(4-amino-3-iodobenzyl)adenosine-5'-(*N*-methyluronamide)] (A_3), respectively.

Table 1

As shown in Table 1, all synthesized 4'-thionucleosides exhibited high binding affinity to the human A_3 receptor in the nanomolar range, while very low binding affinities to A_1 and A_{2A} receptors were observed. Among them, compound **4** displayed a K_i value of 0.28 nM at the A_3 receptor and had the highest affinity among the synthesized compounds. It was selective for A_3 vs rat A_1 and rat A_{2A} receptors by 700- and 23,000-fold, respectively and for A_3 vs human A_1 and human A_{2A} receptors by 4,800- and 36,000-fold, respectively. Compounds **3** and **5** with 3-iodobenzylamino and amino substituents at the N^6 position also exhibited higher binding affinity to the A_3 receptor (K_i = 0.38 and 0.40 nM, respectively) than Cl-IB-MECA. To our best knowledge, it is the first example of a 4'-thionucleoside exhibiting

ultrapotent and selective binding affinity to the A_3 adenosine receptor. Compounds **3**, **4**, and **5** were demonstrated to be full agonists in an assay of human A_3 receptor-mediated inhibition of cyclic AMP in transfected CHO cells.[17] The IC_{50} values were (in nM): 0.21 ± 0.4 (**4**), 0.38 ± 0.6 (**3**), and 1.0 ± 0.3 (**5**).

In conclusion, we have designed and synthesized novel 4'-thioanalogues of Cl-IB-MECA on the basis of the bioisosteric rationale. The final nucleosides were synthesized via the glycosyl donor, 4-thiosugar acetate, which was efficiently synthesized on a preparative scale from D-gulonic γ-lactone (**6**). All synthesized 4'-thionucleosides exhibited extremely high binding affinity to the A_3 receptor with great selectivity to A_1 and A_{2A} receptors, among which 2-chloro-*N*^{6}-methyl-4'-thioadenosine-5'-methyluronamide (**4**) was found to be the most potent and selective agonist at the human A_3 adenosine receptor. It is interesting to note that simple change of furanose to thiofuranose resulted in dramatic increase in binding affinity to the A_3 receptor, but poor binding affinities to the A_1 and A_{2A} receptors. Our results may facilitate the identification of the binding modes of the adenosine receptors. Thorough structure-activity relationship

of this series and molecular modeling study are in progress in our laboratory and will be reported in due course.

Acknowledgement. This research was supported by the grant from the Korea Science and Engineering Foundation (KOSEF 2002-2-21500-001-3).

Supporting Information Available: Complete experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Table 1. Affinities of 4'-thionucleoside analogues in radioligand binding assays at A_1, A_{2A}, and A_3 receptors (human or rat, as indicated).

Compounds	K_i (nM) or % displacement				
	hA_1	rA_1	hA_{2A}	rA_{2A}	hA_3
Cl-IB-MECA (2)	1240 ± 320	820 ± 570	5360 ± 2470	470 ± 365	1.0 ± 0.2
3	193 ± 46	140 ± 43	223 ± 36	348 ± 110	0.38 ± 0.07
4	1330 ± 242	198 ± 14	20% (10 μM)	6340± 90	0.28 ± 0.09
5	89.2± 11.7	294 ± 115	158 ± 29	<10% (1 μM)	0.40 ± 0.06

- All human AR experiments were performed using adherent CHO cells stably transfected with cDNA encoding the appropriate receptor. Binding at rat A_1 and A_{2A}ARs was carried out as using rat brain homogenates. Radioligands: A_1 ([^{3}H]-N^6-(*R*)-phenylisopropyladenosine), A_{2A} ([^{3}H]CGS21680), A_3 ([^{125}I]iodo-AB-MECA).

Supporting Information

N^6-Substituted D-4'-Thioadenosine-5'-methyluronamides: Ultrapotent and Selective Agonists at the Human A_3 Adenosine Receptor

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Synthetic procedure

General. Melting points are uncorrected. ^1H NMR (400 MHz), ^{13}C NMR (100 MHz) and ^{19}F NMR (376 MHz) spectra were measured in $CDCl_3$ and CD_3OD and chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane as internal standard. Elemental analyses were performed at the general instrument laboratory of Ewha Womans University, Korea. TLC was performed on Merck precoated $60F_{254}$ plates. Column chromatography was performed using silica gel 60 (230-400 mesh, Merck). Anhydrous solvents were purified by the standard procedures.

Experimental section

Synthesis

Methanesulfonic acid (*S*)-((4*R*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-((4*S*,5*S*)-5-methanesulfonyloxymethyl-2,2-dimethyl-[1,3]dioxolan-4-yl)-methyl ester (4).

To a stirred slurry of D-gulonic-γ-lactone 6 (20.2 g, 113.5 mmol) and anhydrous $CuSO_4$ (27.0 g, 169.8 mmol) in dry acetone (650 mL) was added conc. H_2SO_4 (1.7 mL), and the mixture was stirred for 24 h at room temperature. The pH of the solution was adjusted to 7 with $Ca(OH)_2$, and the resulting slurry was filtered and evaporated *in vacuo* to afford the diacetonide 7 (28.7 □, 98%) as a light-yellow syrup. It was used in the next step without further purification. To a stirred solution of diacetonide 7 (25.1 g, 97.2 mmol) in ether

(450 mL) was added, cautiously in several portions, lithium aluminum hydride (7.2 □, 190.4 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 10 h. After cooling, the reaction mixture was sequentially treated with water (7.2 mL), 15% aqueous sodium hydroxide solution (7.2 mL), water (21.6 mL), and $MgSO_4$ (19 g), filtered and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1:1) to give the diol (24.1□, 95%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 1.29 (s, 3 H, CH_3), 1.29 (s, 3 H, CH_3), 1.36 (s, 3 H, CH_3), 1.43 (s, 3 H, CH_3), 3.26 (s, 1 H, OH), 3.28 (s, 1 H, OH), 3.36-3.79 (m, 4 H, $HOCH_2$, OCH_2), 3.96-4.03 (m, 2 H, $HOCH_2CH$(OR)R', OC*H*RR'), 4.12-4.22 (m, 2 H); Anal. Calcd for $C_{12}H_{22}O_6$: C, 54.95; H, 8.45. Found: C, 54.67; H, 8.4.

To a stirred solution of diol (4.1 g, 15.7 mmol) in a mixture of dichloromethane (1000 mL) and pyridine (25 mL) was added methanesulfonyl chloride (18.2 mL, 235.5 mmol) at 0 °C. After being stirred at 5 °C for 5 h, the mixture was partitioned between chloroform and saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with chloroform and the combined organic extracts were dried, filtered and evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 2:1) to give the dimesylate **8** (6.4 □, 98%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3 H, CH_3), 1.39 (s, 3 H, CH_3), 1.46 (s, 3 H, CH_3), 1.52 (s, 3 H, CH_3) 3.10 (s, 3 H, SO_2CH_3), 3.18 (s, 3 H, SO_2CH_3), 3.92-4.08 (dd, 1 H, J = 11.7, 7.1 Hz, ROC*H*H), 4.08-4.20 (m, 2 H, ROCH*H*C*H*(OR')), 4.39-4.49 (m, 4 H, $MsOCH_2CH$(OR)C*H*(OR')), 4.81-4.87 (dd, 1 H, J = 6.6, 4.8 Hz, MsOC*H*RR'); Anal. Calcd for $C_{14}H_{26}O_{10}S_2$: C, 40.18; H, 6.26, S, 15.32. Found: C, 40.32; H, 6.11; S, 15.67.

(3a*S*,4*R*,6a*R*)-4-((4*R*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-2,2-dimethyl-tetrahydro-thieno[3,4-d][1,3]dioxole (9).

To a stirred solution of dimesylate **8** (10.1 g, 24.2 mmol) in DMF (260 mL) was added sodium sulfide (8.9 □, 36.3 mmol) and the mixture was heated at 100 °C for 3 h. After the solvent was removed under reduced pressure, the residue was partitioned between ethyl acetate and H_2O. The organic layer was washed with brine and water, dried ($MgSO_4$), filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 5:1) to give **9** (5.9 □, 94%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 3 H, CH_3), 1.34 (s, 3 H, CH_3), 1.44 (s, 3 H, CH_3), 1.52 (s, 3 H, CH_3), 2.88 (d, 1 H, J = 12.7 Hz, 6-C*H*H), 3.09 (td, 1 H, J = 13.1, 2.2 Hz, 6-CH*H*), 3.24 (d, 1 H, J = 8.8 Hz, 4-H), 3.76 (dd, 1 H, J = 8.6, 5.7 Hz, 5'-C*H*H), 3.98 (td, 1 H, J = 9.0, 6.0 Hz, 4'-H). 4.15 (dd, 1 H, J = 8.5, 6.3 Hz, 5'-CH*H*), 4.93 (d, 2 H, J = 1.9 Hz, 3a-H, 6a-H); ^{13}C

NMR ($CDCl_3$) δ 23.8 (CH_3), 24.6 (CH_3), 25.6 (CH_3), 26.1 (CH_3), 36.9 (CH_2), 56.5 (CH), 68.3 (CH_2), 75.6 (CH), 82.3 (CH), 84.5 (CH); FAB-MS m/z 260(M^+); Anal. Calcd for $C_{12}H_{20}O_4S$: C, 55.36; H, 7.74; S, 12.32. Found: C, 55.24; H, 7.72; S, 12.14.

(1*R*)-1-((3a*S*,4*R*,6a*R*)-2,2-Dimethyl-tetrahydro-thieno[3,4-*d*][1,3]dioxol-4-yl)-ethane-1,2-diol (10).

A solution of 9 (5.4 g, 20.1 mmol) in 30% aqueous AcOH (150 mL) was stirred at room temperature for 50 h. The reaction mixture was evaporated under reduced pressure, and the resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1:1) to give the diol **10** (3.4 □, 75%) as a syrup: ^1H NMR ($CDCl_3$) δ 1.34 (s, 3 H, CH_3), 1.53 (s, 3 H, CH_3), 2.90 (dd, 1 H, J = 12.9, 2.2 Hz, 6'-C*H*H), 3.09 (dd, 1 H, J = 12.9, 4.8 Hz, 6'-CH*H*), 3.27 (dd, 1 H, J = 7.8, 1.9 Hz, 4'-H), 3.49 (br s, 2 H, 2×OH), 3.58-3.72 (m, 2 H, HOC*H*HC*H*(OH)), 3.79 (dd, 1 H, J = 11.0, 2.9 Hz, HOCH*H*), 4.93 (m, 2 H, 3a-H, 6a-H); Anal. Calcd for $C_9H_{16}O_4S$: C, 49.07; H, 7.32; S, 14.56. Found: C, 49.24; H, 7.72; S, 14.23.

(3a*S*,4*S*,6a*R*)-2,2-Dimethyl-tetrahydro-thieno[3,4-*d*][1,3]dioxole-4-carbaldehyde (11).

To a stirred solution of diol **10** (2.5 g, 11.2 mmol) in ethyl acetate (50 mL) was added $Pb(OAc)_4$ (5.4 □, 12.3 mmol) at 0 °C and the reaction mixture was stirred for 10 min at which time TLC indicated the absence of starting material. The reaction mixture was filtered and the filtrate was diluted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ solution, dried ($MgSO_4$), and evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 5:1) to give aldehyde 7 (2.1 □, 98%) as a syrup.

^1H NMR ($CDCl_3$) δ 1.33 (s, 3 H, CH_3), 1.52 (s, 3 H, CH_3), 2.61 (dd, 1 H, J = 13.2, 3.9 Hz, 6-C*H*H), 2.87 (dd, 1 H, J = 12.7, 4.1 Hz, 6-CH*H*), 3.93 (s, 1 H, 4-H), 4.92 (t, 1 H, J = 4.1 Hz, 6a-H), 5.10 (d, 1 H, J = 5.4 Hz, 3a-H), 9.43 (s, 1 H, CHO).

To a stirred solution of aldehyde (5.6 g, 30.0 mmol) in MeOH (70 mL) was added, cautiously in several portions, sodium borohydride (1.3 □, 33.6 mmol) at 0 °C. The reaction mixture was stirred for 30 min at the same temperature, before neutralized with glacial AcOH. After evaporation of most of the solvent, the mixture was partitioned between EtOAc (150 mL) and brine (100 mL). The organic layer was dried, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 2:1) to give 11 (5.5 g, 98%) as a syrup: ^1H NMR ($CDCl_3$) δ 1.33 (s, 3 H, CH_3), 1.53 (s, 3 H, CH_3), 2.41 (br s, 1 H, OH), 2.89 (dd, 1 H, J = 12.9, 1.5 Hz, 6-

C*H*H), 3.09 (dd, 1 H, J = 12.7, 4.9 Hz, 6-CH*H*), 3.44 (td, 1 H, J = 6.6, 1.0 Hz, 4-H), 3.59 (d, 2 H, J = 5.4 Hz, HOC*H₂*), 4.71 (dd, 1 H, J = 5.6, 1.2 Hz, 6a-H), 4.91 (td, 1 H, J = 5.3, 1.5 Hz, 3a-H); FAB-MS m/z 190(M^+); Anal. Calcd for $C_8H_{14}O_3S$: C, 50.50; H, 7.42; S, 16.85. Found: C, 50.89; H, 7.72; S, 16.49.

Benzoic acid (3a*S*,4*R*,6a*R*)- 2,2-dimethyl-tetrahydro-thieno[3,4-*d*][1,3]dioxol-4-ylmethyl ester (12).

To a solution of **11** (2.1 g, 11.1 mmol) in pyridine (20 mL) was added benzoyl chloride (1.9 □, 13.4 mmol) at 0 °C and the reaction mixture was stirred for 6 h at room temperature before being quenched with methanol. The reaction mixture was evaporated under reduced pressure. The residue was then taken up in diethyl ether (50 mL), and the pyridinium salt was filtered and washed with diethyl ether. The combined organic layer was concentrated and the resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 4:1) to afford the benzoate **12** (3.2 □, 99%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 3 H, CH_3), 1.47 (s, 3 H, CH_3), 2.87 (dd, 1 H, J = 13.2, 1.5 Hz, 6-C*H*H), 3.11 (dd, 1 H, J = 13.2, 4.9 Hz, 6-CH*H*), 3.57 (m, 1 H, 4-H), 4.25 (dd, 1 H, J = 11.5, 8.0 Hz, BzOC*H*H), 4.35 (dd, 1 H, J = 11.4, 5.8 Hz, BzOCH*H*), 4.72 (dd, 1 H, J = 5.6, 1.2 Hz, 6a-H), 4.91 (td, 1 H, J = 4.4, 1.2 Hz, 3a-H), 7.35-7.99 (m, 5 H, Ph); FAB-MS m/z 294(M^+); Anal. Calcd for $C_{15}H_{18}O_4S$: C, 61.20; H, 6.16; S, 10.89. Found: C, 60.99; H, 6.42; S, 10.77.

Benzoic acid (3a*S*,4*R*,6*R*,6a*R*)- and (3aS,4*R*,6*S*,6a*R*)-6-acetoxy-2,2-dimethyl-tetrahydro-thieno[3,4-*d*][1,3]dioxol-4-ylmethyl ester (14).

To a stirred solution of **12** (1.4 g, 4.6 mmol) in CH_2Cl_2 (30 mL) was added dropwise a solution of m-CPBA (1.0 □, 4.6 mmol, 80%) in CH_2Cl_2 (15 mL) at –78 °C. After being stirred at the same temperature for 45 min, the reaction mixture was quenched by the addition of aqueous saturated $NaHCO_3$ solution. The whole was extracted with CH_2Cl_2, and the organic layer was washed with brine, dried ($MgSO_4$), filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 2:1) to give the sulfoxide **13** (1.4 □, 95%) as a white solid: ^{1}H NMR ($CDCl_3$) of one isomer δ 1.33 (s, 3 H, CH_3), 1.49 (s, 3 H, CH_3), 3.23 (dd, 1 H, J = 14.4, 4.1 Hz, 6-C*H*H), 3.38 (dd, 1 H, J = 14.4, 6.3 Hz, 6-CH*H*), 3.47 (m, 1 H, 4-H), 4.73 (dd, 1 H, J = 11.9, 9.0 Hz, BzOC*H*H), 4.89 (dd, 1 H, J = 11.9, 4.9 Hz, BzOCH*H*), 5.02 (t, 1 H, J = 6.1 Hz, 3a-H), 5.24 (m, 1 H, 6a-H), 7.41-8.03 (m, 5 H, Ph).

A solution of sulfoxide **13** (3.5 g, 11.3 mmol) in Ac_2O (90 mL) was heated at 100 °C for 6 h. After concentration under reduced pressure, the residue was partitioned between ethyl

acetate and H_2O. The organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 3:1) to give acetate **14** (2.5 □, 62%) as a syrup: ^{1}H NMR ($CDCl_3$) of one isomer δ 1.30 (s, 3 H, CH_3), 1.50 (s, 3 H, CH_3), 2.03 (s, 3 H, $COCH_3$), 3.77 (dd, 1 H, J = 9.5, 5.8 Hz, 4-H), 4.37 (dd, 1 H, J = 11.4, 9.8 Hz, *BzOCHH*), 4.23 (dd, 1 H, J = 11.4, 6.1 Hz, BzOCH*H*), 4.94 (d, 1 H, J = 5.6 Hz, 3a-H), 4.98 (d, 1 H, J = 5.6 Hz, 6a-H), 6.06 (s, 1 H, 6-H), 7.42-8.06 (m, 5 H, Ph); FAB-MS m/z 293(M^+-OAc); Anal. Calcd for $C_{17}H_{20}O_6S$: C, 57.94; H, 5.72; S, 9.10. Found: C, 58.14; H, 5.41; S, 9.34.

Benzoic acid (3a*S*,4*R*,6*R*,6a*R*)-6-(2,6-dichloro-purin-9-yl)-2,2-dimethyl-tetrahydro-thieno[3,4-*d*][1,3]dioxol-4-ylmethyl ester (15) and its α-isomer.

A suspension of 2,6-dichloropurine (415 mg, 2.20 mmol), ammonium sulfate (catalytic amount), and HMDS (80 mL) was refluxed for 6 h under nitrogen to provide the silylated derivative. This clear reaction mixture was concentrated to dryness *in vacuo* with keeping exclusion of moisture, and the residue was dissolved in dry 1,2-dichloroethane (15 mL). A solution of acetate **14** (668 m□, 1.90 mmol) in dry 1,2-dichloroethane (20 mL) and TMSOTf (0.42 mL, 2.18 mmol) were added at 0 °C, and the reaction mixture was stirred for 20 min at the same temperature and then refluxed for 4 h, during which time the initially formed N-3 isomer was converted to N-9 isomer. After saturated aqueous $NaHCO_3$ solution (20 mL) was added, the mixture was stirred for 15 min. Two layers were separated, and the aqueous layer was extracted with methylene chloride. The combining organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 2:1) to give β-anomer **15** (409 mg, 44%) as a white foam and its α-anomer (102 mg, 11%) as a white foam.

β-anomer **15**: UV (MeOH) λ_{max} 269 nm (pH 7); ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 3 H, CH_3), 1.64 (s, 3 H, CH_3), 4.11 (td, 1 H, J = 6.8, 2.7 Hz, 4-H), 4.57 (dd, 1 H, J = 11.4, 6.6 Hz, BzOC*H*H), 4.75 (dd, 1 H, J = 11.4, 7.1 Hz, BzOC*H*H), 5.19 (dd, 1 H, J = 5.4, 2.7 Hz, 3a-H), 5.40 (dd, 1 H, J = 5.4, 1.9 Hz, 6a-H), 6.10 (d, 1 H, J = 1.9 Hz, 6-H), 7.37-7.97 (m, 5 H, Ph), 8.38 (s, 1 H, H-8); FAB-MS m/z 482(M^++1); Anal. Calcd for $C_{20}H_{18}Cl_2N_4O_4S$: C, 49.90; H, 3.77; N, 11.64; S, 6.66. Found: C, 49.53; H, 3.42; N, 11.93; S, 6.36.

α-anomer: UV (MeOH) λ_{max} 279 nm (pH 7); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3 H, CH_3), 1.64 (s, 3 H, CH_3), 4.08 (m, 1 H, 4-H), 4.51 (dd, 1 H, J = 11.7, 6.3 Hz, BzOC*H*H), 4.62 (dd, 1 H,

J = 11.7, 7.1 Hz, BzOCH*H*), 5.01 (m, 1 H, 3a-H), 5.11 (m, 1 H, 6a-H), 6.50 (d, 1 H, J = 2.2 Hz, 6-H), 7.37-7.99 (m, 5 H, Ph), 8.87 (s, 1 H, H-8); FAB-MS m/z 482(M^++1); Anal. Calcd for $C_{20}H_{18}C_{12}N_4O_4S$: C, 49.90; H, 3.77; N, 11.64; S, 6.66. Found: C, 49.57; H, 4.01; N, 11.92; S, 6.58.

Benzoic acid (3a*S*,4*R*,6*R*,6a*R*)-6-[2-chloro-6-(3-iodo-benzylamino)-purin-9-yl]-2,2-dimethyl-tetrahydro-thieno[3,4-*d*][1,3]dioxol-4-ylmethyl ester (17).

A solution of 15 (254 mg, 0.53 mmol), 3-iodobenzylamine hydrochloride (200 mg, 0.68 mmol), and triethylamine (0.22 mL, 1.53 mmol) in ethanol (1.5 mL) was stirred at room temperature for 3 d. The reaction mixture was evaporated under reduced pressure, and the resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1:1) to give 17 (322 m□, 90%) as a white foam: UV (MeOH) λmax 272 nm (pH 7); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3 H, CH_3), 1.63 (s, 3 H, CH_3), 4.11 (td, 1 H, J = 7.3, 2.7 Hz, 4-H), 4.57 (dd, 1 H, J = 11.4, 6.8 Hz, BzOC*H*H), 4.76 (m, 3 H, BzOCH*H*, N-CH_2), 5.22 (dd, 1 H, J = 5.6, 2.7 Hz, 3a-H), 5.38 (dd, 1 H, J = 5.6, 1.9 Hz, 6a-H), 6.01 (d, 1 H, J = 1.9 Hz, 6-H), 6.28 (br s, 1 H, NH), 7.04-8.02 (m, 10 H, aromatic H); FAB-MS m/z 679(M^++1); Anal. Calcd for $C_{27}H_{25}ClIN_5O_4S$: C, 47.83; H, 3.72; N, 10.33; S, 4.73. Found: C, 47.50; H, 3.48; N, 10.69; S, 4.48.

Benzoic acid (3a*S*,4*R*,6*R*,6a*R*)-6-(2-chloro-6-methylamino-purin-9-yl)-2,2-dimethyl-tetrahydro-thieno[3,4-*d*][1,3]dioxol-4-ylmethyl ester (18).

A solution of 15 (454 mg, 0.94 mmol) in CH_3NH_2 (20 mL, 40 mmol, 2 *N* THF solution) was stirred at room temperature in a sealed tube for 24 h. After the reaction mixture was concentrated to dryness, the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1:1) to give 18 (383 m□, 85%) as a white foam: UV (MeOH) λmax 271 nm (pH 7); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3 H, CH_3), 1.63 (s, 3 H, CH_3), 3.02 (d, 3 H, J = 4.9 Hz, N-CH_3), 4.01 (td, 1 H, J = 7.8, 2.7 Hz, 4-H), 4.57 (dd, 1 H, J = 11.5, 6.8 Hz, BzOC*H*H), 4.75 (dd, 1 H, J = 11.5, 7.8 Hz, BzOCH*H*), 5.23 (dd, 1 H, J = 5.6, 2.9 Hz, 3a-H), 5.38 (dd, 1 H, J = 5.6, 1.9 Hz, 6a-H), 6.01 (d, 1 H, J = 1.9 Hz, 6-H), 6.16 (br s, 1 H, NH), 7.40-8.02 (m, 6 H, Ph, H-8); FAB-MS m/z 477 (M^++1); Anal. Calcd for $C_{21}H_{22}ClN_5O_4S$: C, 52.99; H, 4.66; N, 14.71; S, 6.74. Found: C, 52.71; H, 4.49; N, 14.76; S, 6.64.

Benzoic acid (3a*S*,4*R*,6*R*,6a*R*)-6-(6-amino-2-chloro-purin-9-yl)-2,2-dimethyl-tetrahydro-thieno[3,4-*d*][1,3]dioxol-4-yl methyl ester (19).

A solution of 15 (454 mg, 0.94 mmol) in saturated ethanolic ammonia (25 mL) was heated at 50 °C in a sealed tube for 24 h. The reaction mixture was evaporated to dryness under

reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1:1) to afford **19** (384 mg, 88%) as a white foam: UV (MeOH) λ max 264nm (pH 7); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3 H, CH_3), 1.63 (s, 3 H, CH_3), 4.01 (td, 1 H, J = 7.3, 2.7 Hz, 4-H), 4.57 (dd, 1 H, *J* = 11.5, 6.8 Hz, BzOC*H*H), 4.77 (dd, 1 H, *J* = 11.5, 7.6 Hz, BzOCH*H*), 5.23 (dd, 1 H, *J* = 5.6, 2.7 Hz, 3a-H), 5.38 (dd, 1 H, *J* = 5.6, 1.9 Hz, 6a-H), 6.02 (d, 1 H, *J* = 1.9 Hz, 6-H), 6.29 (br s, 2 H, NH_2), 7.40-8.02 (m, 6 H, Ph, H-8); FAB-MS m/z 463(M^++1); Anal. Calcd for $C_{20}H_{20}ClN_5O_4S$: C, 52.00; H, 4.36; N, 15.16; S, 6.94. Found: C, 52.32; H, 4.42; N, 14.89; S, 6.71.

{(2*R*,3*S*,4*R*,5*R*)-3,4-Bis-(*tert*-butyl-dimethyl-silanyloxy)-5-[2-chloro-6-(3-iodo-benzylamino)-purin-9-yl]-tetrahydro-thiophen-2-yl}-methanol (20).

A solution of **17** (122 mg, 0.18 mmol) in 80% aqueous AcOH solution (6 mL) was stirred at 55 °C for 12 h. The solvent was removed under reduced pressure. After neutralized with methanolic ammonia, the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 15:1) to give diol (73 mg, 64%) as a white foam. To a stirred solution of diol (450 mg, 0.71 mmol) and imidazole (286 mg, 4.20 mmol) in dry DMF (15 mL) was added dropwise a solution of *tert*-butylchlorodimethylsilane (316 mg, 2.10 mmol) in dry DMF (5 mL) and the reaction mixture was stirred at 50 °C for 24 h. The mixture was partitioned between CH_2Cl_2 and water, and the organic layer was washed with water, aqueous $NaHCO_3$ solution, water, and brine, and dried ($MgSO_4$). Filteration and evaporation of the mixture under reduced pressure gave the crude disilyl ether, which was used in the next step without further purification. To a stirred solution of disilyl ether in methanol (15 mL) was added sodium methoxide (90 mg, 1.74 mmol) and the mixture was stirred at room temperature for 4 h. After being neutralized with glacial acetic acid, the mixture was evaporated under reduced pressure to give the resulting residue, which was purified by silica gel column chromatography (hexane/ethyl acetate = 2:1) to give **20** (290 mg, 54%) as a white solid: UV (MeOH) λ_{max} 272 nm (pH 7); ^{1}H NMR ($CDCl_3$) δ 0.01 (m, 12 H, 4×Si-CH_3), 0.80 (s, 9 H, $C(CH_3)_3$), 0.85 (s, 9 H, $C(CH_3)_3$), 3.29 (dd, 1 H, *J* = 11.4, 5.8 Hz, 2-H), 4.24 (m, 1 H, HOC*H*H), 4.47 (dd, 1 H, *J* = 11.9, 5.8 Hz, 3-H), 4.73 (m, 3 H, HOCH*H*, N-CH_2), 4.86 (dd, 1 H, *J* = 11.9, 6.3 Hz, 4-H), 5.67 (d, 1 H, *J* = 4.6 Hz, 5-H), 6.15 (br t, 1 H, exchangeable with D_2O, OH), 7.02 (t, 1 H, *J* = 7.7 Hz, 5'-H), 7.27 (d, 1 H, *J* = 7.5 Hz, 6'-H), 7.43 (d, 1 H, *J* = 7.8 Hz, 4'-H), 7.59 (br s, 1 H, exchangeable with D_2O, NH), 7.67 (s, 1 H, 2'-H), 8.03 (s, 1 H, H-8); FAB-MS m/z 763 (M^++1); Anal. Calcd for $C_{29}H_{45}ClIN_5O_3SSi_2$: C, 45.69; H, 5.95; N, 9.19; S, 4.21. Found: C, 45.47; H, 5.92; N, 8.97;

S, 4.39.

[(2*R*,3*S*,4*R*,5*R*)-3,4-Bis-(*tert*-butyl-dimethyl-silanyloxy)-5-(2-chloro-6-methylamino-purin-9-yl)-tetrahydro-thiophen-2-yl]-methanol (21).

Compound **15** (105 mg, 0.22 mmol) was converted to diol (65 mg, 68%) as a white foam. Diol (160 mg, 0.38 mmol) was converted to **21** (112 mg, 54%) as a white solid according to the same procedure used in the synthesis of **20**: UV (MeOH) λ_{max} 269 nm (pH 7); ^{1}H NMR ($CDCl_3$) δ 0.01 (m, 12 H, 4×Si-CH_3), 0.81 (s, 9 H, C(CH_3)$_3$), 0.86 (s, 9 H, C(CH_3)$_3$), 3.13 (br s, 3 H, N-CH_3), 3.72 (dd, 1 H, *J* = 11.7, 5.8 Hz, 2-H), 4.24 (m, 1 H, 3-H), 4.48 (dd, 1 H, *J* = 11.7, 5.8 Hz, HOC*H*H), 4.75 (m, 1 H, 4-H), 4.87 (dd, 1 H, *J* = 11.7, 6.3 Hz, HOCH*H*), 5.67 (d, 1 H, *J* = 4.6 Hz, 5-H), 5.88 (br s, 1 H, exchangeable with D_2O, OH), 8.00 (s, 1 H, H-8), 8.03 (br s, 1 H, exchangeable with D_2O, NH).

[(2*R*,3*S*,4R,5*R*)-5-(6-Amino-2-chloro-purin-9-yl)-3,4-bis-(*tert*-butyl-dimethyl-silanyloxy)-tetrahydro-thiophen-2-yl]-methanol (22).

Compound **15** (102 mg, 0.22 mmol) was converted to diol (63 mg, 68%) as a white foam. Diol (230 mg, 0.55 mmol) was converted to **22** (169 mg, 56%) as a white solid according to the same procedure used in the synthesis of **20**: UV (MeOH) λ_{max} 263nm (pH 7); ^{1}H NMR ($CDCl_3$) δ 0.01 (m, 12 H, 4×Si-CH_3), 0.79 (s, 9 H, C(CH_3)$_3$), 0.83 (s, 9 H, C(CH_3)$_3$), 3.41 (dd, 1 H, *J* = 11.2, 4.7 Hz, 2-H), 4.21 (m, 1 H, HOC*H*H), 4.56 (dd, 1 H, *J* = 10.9, 4.7 Hz, 3-H), 4.73 (m, 1 H, HOCH*H*), 4.86 (dd, 1 H, *J* = 10.9, 5.8 Hz, 4-H), 5.71 (d, 1 H, *J* = 5.8 Hz, 5-H), 6.20 (br d, 1 H, exchangeable with D_2O, OH), 7.61 (br s, 2 H, exchangeable with D_2O, NH_2), 8.12 (s, 1 H, H-8).

(2*S*,3*S*,4*R*,5*R*)-5-[2-Chloro-6-(3-iodo-benzylamino)-purin-9-yl]-3,4-dihydroxy-tetrahydro-thiophene-2-carboxylic acid methylamide (3).

To a stirred solution of **20** (228 mg, 0.30 mmol) in dry DMF (20 mL) was added pyridinium dichromate (2.6 g, 6.91 mmol) and the reaction mixture was stirred at room temperature for 20 h. After being poured into water (30 mL), the reaction mixture was extracted with EtOAc (100 mL×5). The organic layer was dried ($MgSO_4$), and evaporated to give the crude carboxylic acid, which was dried for 2 d under vacuum and used in the next reaction without further purification. A suspension of the crude carboxylic acid, dimethyl sulfate (1 mL, 10.57 mmol) and K_2CO_3 (100 mg, 0.72 mmol) in acetone (6 mL) was stirred at room temperature for 2 h. After the reaction mixture was evaporated, the residue was dissolved in ethyl acetate, washed with water and brine, dried ($MgSO_4$), and evaporated to give methyl ester (200 mg), which was used in the next step without further

purification. A stirred solution of crude methyl ester (200 mg) in methylamine (20 mL, 40.0 mmol, 2 *N* THF solution) was heated at 50 °C for 24 h in a sealed tube. The volatiles were removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1:2) to give silyl amide (99 mg, 42%) as a white foam: UV (MeOH) λ_{max} 272 nm (pH 7); ^{1}H NMR ($CDCl_3$) δ 0.01 (m, 12 H, 4×Si-CH_3), 0.70 (s, 9 H, $C(CH_3)_3$), 0.84 (s, 9 H, $C(CH_3)_3$), 2.70 (d, 3 H, J = 4.7 Hz, N-CH_3), 3.81 (d, 1 H, J = 4.7 Hz, 2-H), 4.39 (m, 1 H, 3-H), 4.55 (m, 1 H, 4-H), 4.61 (d, 2 H, J = 5.7 Hz, N-CH_2), 5.83 (d, 1 H, J = 5.4 Hz, 5-H), 7.15 (t, 1 H, J = 7.7 Hz, 5'-H), 7.36 (d, 1 H, J = 7.5 Hz, 6'-H), 7.61 (d, 1 H, J = 7.8 Hz, 4'-H), 7.76 (s, 1 H, 2'-H), 8.33 (br s, 1 H, exchangeable with D_2O, NH), 8.61 (s, 1 H, H-8), 9.00 (br s, 1 H, exchangeable with D_2O, NH); FAB-MS m/z 790 (M^++1); Anal. Calcd for $C_{30}H_{46}ClIN_6O_3SSi_2$: C, 45.65; H, 5.87; N, 10.65; S, 4.06. Found: C, 45.34; H, 5.55; N, 10.37; S, 4.34.

To a stirred solution of silyl amide (75 mg, 0.09 mmol) in THF (5 mL) was added TBAF (0.25 mL, 0.25 mmol, 1M THF solution) and the reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated and the resulting residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 7:1) to give **3** (33 mg, 63%) as a white solid: mp 140-141 °C; $[\alpha]^{25}_D$ -19.5° (c 0.32, MeOH); UV (MeOH) λ_{max} 272 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 2.69 (d, 3 H, J = 4.5 Hz, N-CH_3), 3.82 (d, 1 H, J = 4.6 Hz, 2-H), 4.37 (br dd, 1 H, J = 8.4, 4.6 Hz, 3-H), 4.55 (m, 1 H, 4-H), 4.60 (d, 2 H, J = 5.7 Hz, N-CH_2), 5.59 (d, 1 H, J = 5.5 Hz, exchangeable with D_2O, OH), 5.78 (d, 1 H, J = 5.1 Hz, exchangeable with D_2O, OH), 5.83 (d, 1 H, J = 5.4 Hz, 5-H), 7.13 (t, 1 H, J = 7.8 Hz, 5'-H), 7.35 (d, 1 H, J = 7.6 Hz, 6'-H), 7.60 (d, 1 H, J = 7.8 Hz, 4'-H), 7.75 (s, 1 H, 2'-H), 8.32 (br q, 1 H, exchangeable with D_2O, NH), 8.60 (s, 1 H, H-8), 8.99 (br t, 1 H, J = 6.1 Hz, exchangeable with D_2O, NH); ^{13}C NMR (DMSO-d_6) δ 42.5 (CH), 51.8 (CH_2), 59.7 (CH), 62.6 (CH), 75.4 (CH_3), 78.2 (CH), 118.4 (C), 126.8 (CH), 130.5 (CH), 135.5 (CH), 136.0 (CH), 140.3 (C), 141.8 (C), 149.9 (C), 153.0 (C), 154.7 (CH), 170.3 (C), 170.7 (C); FAB-MS m/z 561 (M^+); Anal. Calcd for $C_{18}H_{18}ClIN_6O_3S$: C, 38.55; H, 3.24; N, 14.99; S, 5.72. Found: C, 38.45; H, 3.40; N, 14.97; S, 5.61.

(2*S*,3*S*,4*R*,5*R*)-5-(2-Chloro-6-methylamino-purin-9-yl)-3,4-dihydroxy-tetrahydro-thiophene-2-carboxylic acid methylamide (4).

Compound 21 (105 mg, 0.19 mmol) was converted to the silyl amide (48 mg, 44%) as a white foam according to the same procedure used in the synthesis of 3: UV (MeOH) λ_{max} 270 nm (pH 7); ^{1}H NMR ($CDCl_3$) δ 0.01 (m, 12 H, 4×Si-CH_3), 0.82 (s, 9 H, $C(CH_3)_3$),

0.85 (s, 9 H, $C(CH_3)_3$), 3.21 (br s, 3 H, N-CH_3), 3.21 (br s, 3 H, N-CH_3), 3.75 (d, 1 H, J = 4.8 Hz, 2-H), 4.28 (m, 1 H, 3-H), 4.65 (m, 1 H, 4-H), 5.77 (d, 1 H, J = 4.6 Hz, 5-H), 7.12 (br s, 1 H, exchangeable with D_2O, NH), 8.03 (br s, 1 H, exchangeable with D_2O, NH), 8.44 (s, 1 H, H-8).

Silyl amide (48 mg, 0.08 mmol) was converted to compound **4** (19 mg, 65%) as a white solid according to the same procedure used in the synthesis of **3**.

Compound **4**: mp 139-141 °C; $[\alpha]^{19}_D$ -22.0° (c 0.13, MeOH); UV (MeOH) λ_{max} 270 nm (pH 7); ^1H NMR (DMSO-d_6) δ 2.69 (d, 3 H, J = 4.5 Hz, N-CH_3), 2.92 (d, 3 H, J = 4.0 Hz, N-CH_3), 3.81 (d, 1 H, J = 4.6 Hz, 2-H), 4.36 (br dd, 1 H, J = 8.5, 4.8 Hz, 3-H), 4.52 (br dd, 1 H, J = 8.5, 5.0 Hz, 4-H), 5.61 (d, 1 H, J = 5.4 Hz, exchangeable with D_2O, OH), 5.78 (d, 1 H, J = 5.1 Hz, exchangeable with D_2O, OH), 5.82 (d, 1 H, J = 5.5 Hz, 5-H), 8.33 (br q, 2 H, exchangeable with D_2O, NH, NH), 8.54 (s, 1 H, H-8); ^{13}C NMR (DMSO-d_6) δ 51.8 (CH_3), 55.5 (CH), 62.6 (CH), 69.6 (CH), 75.4 (CH_3), 78.2 (CH), 118.5 (C), 139.9 (C), 149.5 (C), 153.3 (C), 155.5 (CH), 170.7 (C); FAB-MS m/z 359(M^+); Anal. Calcd for $C_{12}H_{15}ClN_6O_3S$: C, 40.17; H, 4.21; N, 23.42; S, 8.94. Found: C, 40.21; H, 4.42; N, 23.57; S, 8.98.

(2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-2-chloro-purin-9-yl)-3,4-dihydroxy-tetrahydro-thiophene-2-carboxylic acid methylamide (5).

Compound **22** (150 mg, 0.27 mmol) was converted to the silyl amide (80 mg, 51%) as a white foam according to the same procedure used in the synthesis of **3**: UV (MeOH) λ_{max} 264 nm (pH 7); ^1H NMR ($CDCl_3$) δ 0.01 (m, 12 H, 4×Si-CH_3), 0.79 (s, 9 H, $C(CH_3)_3$), 0.85 (s, 9 H, $C(CH_3)_3$), 2.67 (d, 3 H, J = 4.1 Hz, N-CH_3), 3.78 (d, 1 H, J = 4.8 Hz, 2-H), 4.35 (m, 1 H, 3-H), 4.57 (m, 1 H, 4-H), 5.70 (d, 1 H, J = 5.6 Hz, 5-H), 7.72 (br s, 2 H, exchangeable with D_2O, NH_2), 7.72 (br s, 1 H, exchangeable with D_2O, NH), 8.10 (s, 1 H, H-8).

Silyl amide (140 mg, 0.24 mmol) was converted to compound **5** (58 m□, 69%) as a white solid according to the same procedure used in the synthesis of **3**: mp 233-235 °C; $[\alpha]^{26}_D$ -20.2 (c 0.1, MeOH); UV (MeOH) λ_{max} 264 nm (pH 7); ^1H NMR (DMSO-d_6) δ 2.70 (d, 3 H, J = 4.1 Hz, N-CH_3), 3.82 (d, 1 H, J = 4.6 Hz, 2-H), 4.36 (dd, 1 H, J = 8.7, 4.6 Hz, 3-H), 4.53 (m, 1 H, 4-H), 5.60 (d, 1 H, J = 5.4 Hz, exchangeable with D_2O, OH), 5.78 (d, 1 H, J = 5.1 Hz, exchangeable with D_2O, OH), 5.82 (d, 1 H, J = 5.5 Hz, 5-H), 7.87 (br s, 2 H, exchangeable with D_2O, NH_2), 8.33 (br q, 1 H, exchangeable with D_2O, NH), 8.55 (s, 1 H, H-8); ^{13}C NMR (DMSO-d_6) δ 51.8 (CH), 57.5 (CH), 62.5 (CH_3), 75.4 (CH), 78.1 (CH),

118.4 (C), 140.1 (C), 150.5 (C), 153.0 (C), 156.7 (CH), 170.7 (C); FAB-MS m/z 345(M^+); Anal. Calcd for $C_{11}H_{13}ClN_6O_3S$: C, 38.32; H, 3.80; N, 24.38; S, 9.30. Found: C, 38.70; H, 3.75; N, 24.57; S, 9.62.

Pharmacological Methods

[^{125}I]N^6-(4-amino-3-iodobenzyl)adenosine-5'-*N*-methyluronamide (I-AB-MECA; 2000 Ci/mmol), [^{3}H]8-ethyl-4-methyl-2-phenyl-(8R)-4,5,7,8-tetrahydro-1H-imidazo[2,1-i]-purin-5-one (PSB-11, 53 Ci/mmol) and [^{3}H]cyclic AMP (40 Ci/mmol) were from Amersham Pharmacia Biotech (Buckinghamshire, UK).

Cell culture and membrane preparation

CHO (Chinese hamster ovary) cells expressing recombinant the human A_3AR were cultured in DMEM supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, 2 μmol/mL glutamine and 800 μg/mL geneticin. The CHO cells expressing rat A_3ARs were cultured in DMEM and F12 (1:1). Cells were harvested by trypsinization. After homogenization and suspension, cells were centrifuged at 500 *g* for 10 min, and the pellet was re-suspended in 50 mM Tris·HCl buffer (pH 8.0) containing 10 mM $MgCl_2$, 1 mM EDTA and 0.1 mg/mL CHAPS. The suspension was homogenized with an electric homogenizer for 10 sec, and was then re-centrifuged at 20,000 *g* for 20 min at 4°C. The resultant pellets were resuspended in buffer in the presence of 3 Units/mL adenosine deaminase, and the suspension was stored at -80°C until the binding experiments. Striatal and forebrain tissues from Wistar rats were homogenized in ice-cold 50 mM Tris·HCl buffer, pH 7.4, using an electric homogenizer. The homogenate was centrifuged at 20,000 *g* for 10 min at 4°C, and the pellet was washed in fresh buffer. The final pellet was stored at -80°C until the binding experiments. The protein concentration was measured using the Bradford assay (Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 1976, *72*, 248-254).

Binding assay using [^{125}I]4-amino-3-iodobenzyl)adenosine-5'-N-methyluronamide

For competitive binding assay, each tube contained 50 μL membrane suspension (20 μg protein), 25 μl [^{125}I]4-amino-3-iodobenzyl)adenosine-5'-*N*-methyluronamide (1.0 nM), and 25 μL of increasing concentrations of the test ligands in Tris·HCl buffer (50 mM, pH 8.0) containing 10 mM $MgCl_2$, 1 mM EDTA. Nonspecific binding was determined using 10

μM of **12** in the buffer. The mixtures were incubated at 37°C for 60 min. Binding reactions were terminated by filtration through Whatman GF/B filters under reduced pressure using a MT-24 cell harvester (Brandell, Gaithersburgh, MD, USA). Filters were washed three times with 9 mL ice-cold buffer. Radioactivity was determined in a Beckman 5500B γ-counter.

Cyclic AMP accumulation assay

Intracellular cyclic AMP levels were measured with a competitive protein binding method (Nordstedt, C.; Fredholm, B. B. A modification of a protein-binding method for rapid quantification of cAMP in cell-culture supernatants and body fluid. *Anal. Biochem.* **1990**, *189*, 231-234; Post S. R.; Ostrom R. S.; Insel P. A. Biochemical methods for detection and measurement of cyclic AMP and adenylyl cyclase activity. *Methods Mol. Biol.* **2000**, *126*, 363-374). CHO cell that expressed recombinant human and rat A_3ARs were harvested by trypsinization. After centrifugation and resupended in medium, cells were planted in 24-well plates in 1.0 mL medium. After 24 h, the medium was removed and cells were washed three times with 1 mL DMEM, containing 50 mM HEPES, pH 7.4. Cells were then treated with agonists and/or test compounds in the presence of rolipram (10 μM) and adenosine deaminase (3 units/mL). After 45 min forskolin (10 μM) was added to the medium, and incubation was continued an additional 15 min. The reaction was terminated by removing the supernatant, and cells were lysed upon the addition of 200 μL of 0.1 M ice-cold HCl. The cell lysate was resuspended and stored at -20°C. For determination of cyclic AMP production, protein kinase A (PKA) was incubated with [^{3}H]cyclic AMP (2 nM) in K_2HPO_4/EDTA buffer (K_2HPO_4, 150 mM; EDTA, 10 mM), 20 μL of the cell lysate, and 30 μL 0.1 M HCl or 50 μL of cyclic AMP solution (0-16 pmol/200 μL for standard curve). Bound radioactivity was separated by rapid filtration through Whatman GF/C filters and washed once with cold buffer. Bound radioactivity was measured by liquid scintillation spectrometry.

Summary

Abstract

The present invention relates to a thionucleoside derivative compound with a novel chemical structure that can control cancer and inflammatory diseases, and the pharmacuetical compositions containing this compound. In the present invention, a ligand that selectively activates the adenosine A_3 receptor was constructed and examined for pharmacological effect to provide a pharmaceutical composition useful for the prevention and treatment of cancer and inflammatory diseases.

Key words:

Cancer, Inflammation, Thionucleoside, Adenosine, Receptor, Ligand, Pharmaceutical composition

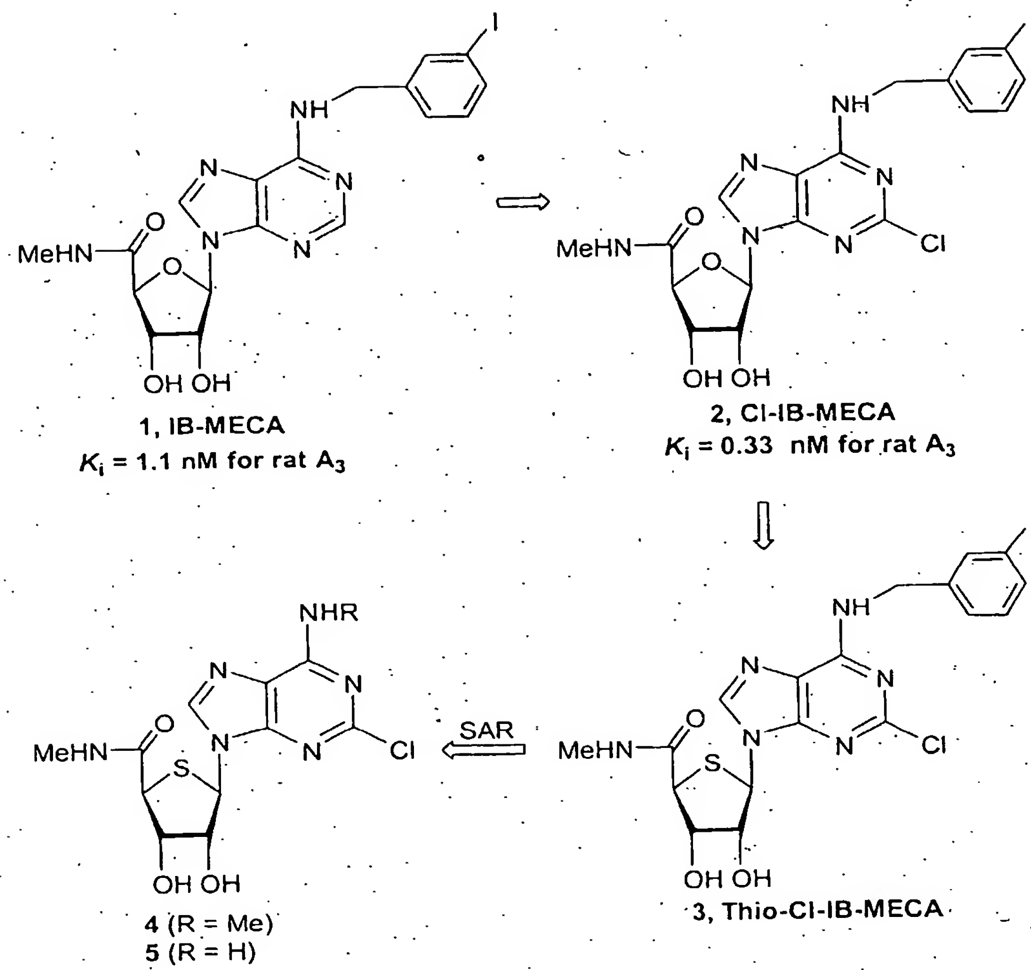
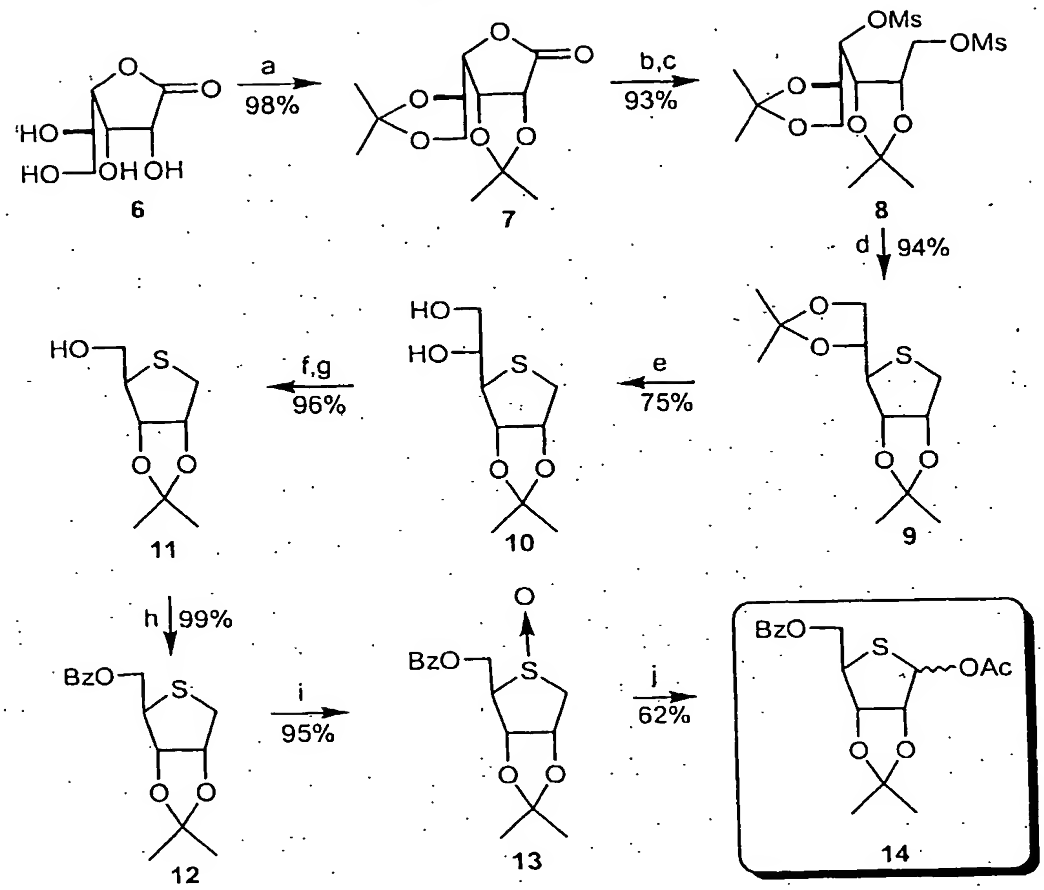


Figure 1. The rationale for the design of the target 4'-thionucleosides.

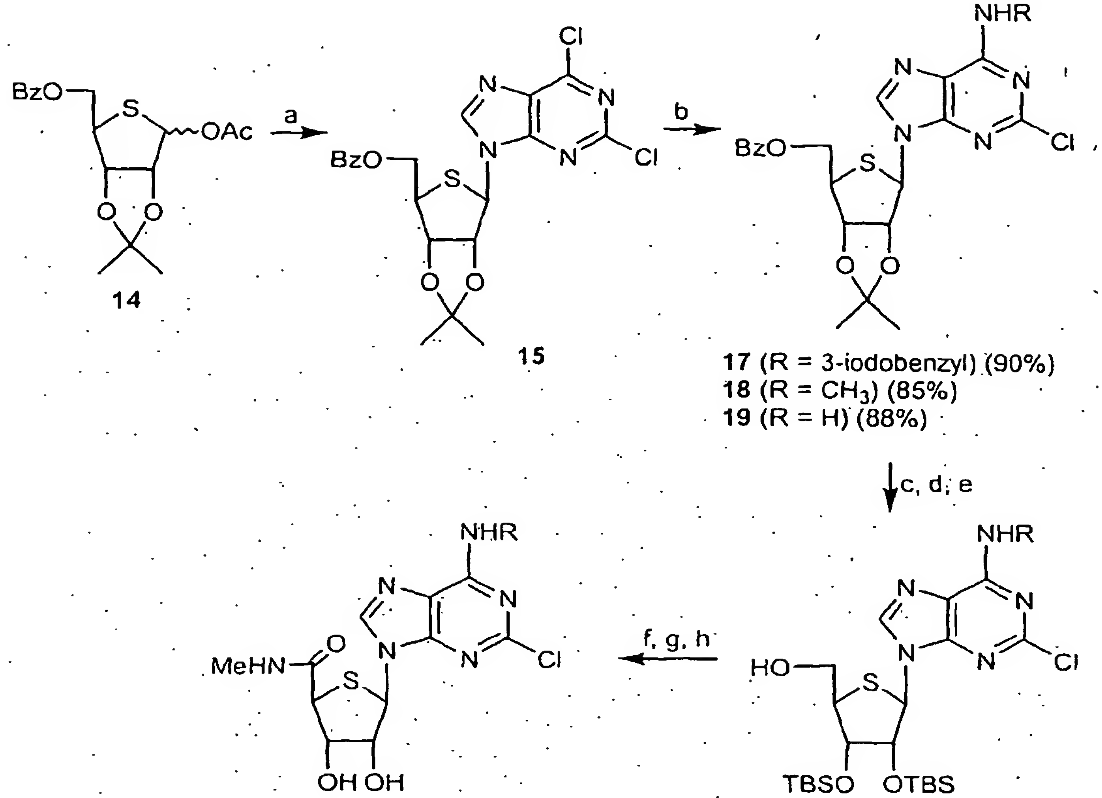
Scheme 1



Reagents: a) CH_3COCH_3, H_2SO_4, $CuSO_4$, rt; b) $LiAlH_4$, ether; c) MsCl, Et_3N, CH_2Cl_2; d) Na_2S, DMF, heat; e) 30% AcOH; f) $Pb(OAc)_4$, EtOAc; g) $NaBH_4$, MeOH; h) BzCl, pyridine; i) *m*CPBA, CH_2Cl_2; j) Ac_2O.

Fig. 2

Scheme 2



14

15

17 (R = 3-iodobenzyl) (90%)
18 (R = CH_3) (85%)
19 (R = H) (88%)

20 (R = 3-iodobenzyl) (35%)
21 (R = CH_3) (37%)
22 (R = H) (38%)

3 (R = 3-iodobenzyl) (27%)
4 (R = CH_3) (29%)
5 (R = H) (36%)

Reagents: a) silylated 2,6-dichloropurine, TMSOTf; b) RNH_2; c) 80% AcOH; d) TBSCl, DMF; e) NaOMe, MeOH; f) i. PDC, DMF, ii. K_2CO_3, $(CH_3O)_2SO_2$; g) 40% $MeNH_2$, MeOH; h) n-Bu_4NF, THF.

Fig. 3

Application Data Sheet

APPLICATION INFORMATION

Application Number::	
Filing Date::	
Application Type::	Provisional
Subject Matter::	Utility
Suggested classification::	
Suggested Group Art Unit::	
CD-ROM or CD-R?::	None
Number of CD Disks:	
Number of Copies of CDs::	
Sequence Submission?::	
Computer Readable From (CRF)?::	No
Number of Copies of CRF::	
Title::	THIONUCLEOSIDE DERIVATIVES HAVING POTENT ANTI-CANCER ACTIVITY AND PHARMACEUTICAL COMPOSITIONS CONTAINING THE SAME
Attorney Docket Number::	402690
Request for Early Publication?::	No
Request for Non-Publication?::	No
Suggested Drawing Figure::	
Total Drawing Sheets::	3
Small Entity?::	No
Latin Name::	
Variety denomination name::	
Petition Included?::	No
Petition Type::	
Licensed US Govt. Agency::	National Institutes of Health
Contract or Grant Numbers::	
Secrecy Order in Parent Appl.?::	No

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Representative Customer Number:: 23548

Representative Designation::	Registration Number::	Representative Name::

DOMESTIC PRIORITY INFORMATION

Application::	Continuity Type::	Parent Application::	Parent Filing Date::

FOREIGN APPLICATION INFORMATION

Country::	Application Number::	Filing Date::	Priority Claimed

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

INVENTOR(S)

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☐ *Additional inventors are being named on the separately numbered sheets attached hereto.*

TITLE OF THE INVENTION (280 characters max)

THIONUCLEOSIDE DERIVATIVES HAVING POTENT ANTI-CANCER ACTIVITY AND PHARMACEUTICAL COMPOSITIONS CONTAINING THE SAME

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ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification *Number of Pages:* 81
(including any claims and abstract)
☒ Drawings *Number of Sheets:* 3
☒ Application Data Sheet. See 37 CFR 1.76

☐ Power of Attorney
☐ Assignment
☐ CD(s), Number
☐ Other (specify)

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27.
☐ A check or money order is enclosed to cover the filing fee(s).
☒ The Commissioner is hereby authorized to charge filing fee(s) or credit any overpayment to Deposit Account Number 12-1216. A duplicate copy of this communication is enclosed for that purpose.
☒ The Commissioner is hereby authorized to charge any deficiencies in filing fees to Deposit Account Number 12-1216. A duplicate copy of this communication is enclosed for that purpose.

FILING FEE AMOUNT: $160.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government:
☐ No.
☒ Yes, the name of the U.S. Government agency and the Government contract number are: National Institutes of Health

Respectfully submitted,

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SEND TO: Mail Stop Patent Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450

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